Published in final edited form as:
Physiol Rev. 2012 October ; 92(4): 1515–1542. doi:10.1152/physrev.00047.2011.

S-ADENOSYLMETHIONINE IN LIVER HEALTH, INJURY, AND CANCER

Shelly C. Lu and **José M. Mato**
Division of Gastroenterology and Liver Diseases, USC Research Center for Liver Diseases, Southern California Research Center for ALPD and Cirrhosis, Keck School of Medicine, Los Angeles, California; and CIC bioGUNE, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Technology, Park of Bizkaia, Derio, Bizkaia, Spain

Abstract

S-adenosylmethionine (AdoMet, also known as SAM and SAMe) is the principal biological methyl donor synthesized in all mammalian cells but most abundantly in the liver. Biosynthesis of AdoMet requires the enzyme methionine adenosyltransferase (MAT). In mammals, two genes, *MAT1A* that is largely expressed by normal liver and *MAT2A* that is expressed by all extrahepatic tissues, encode MAT. Patients with chronic liver disease have reduced MAT activity and AdoMet levels. Mice lacking *Mat1a* have reduced hepatic AdoMet levels and develop oxidative stress, steatohepatitis, and hepatocellular carcinoma (HCC). In these mice, several signaling pathways are abnormal that can contribute to HCC formation. However, injury and HCC also occur if hepatic AdoMet level is excessive chronically. This can result from inactive mutation of the enzyme glycine *N*-methyltransferase (GNMT). Children with GNMT mutation have elevated liver transaminases, and *Gnmt* knockout mice develop liver injury, fibrosis, and HCC. Thus a normal hepatic AdoMet level is necessary to maintain liver health and prevent injury and HCC. AdoMet is effective in cholestasis of pregnancy, and its role in other human liver diseases remains to be better defined. In experimental models, it is effective as a chemopreventive agent in HCC and perhaps other forms of cancer as well.

I. INTRODUCTION

The discovery of *S*-adenosyl-L-methionine (AdoMet, also frequently abbreviated as SAM and SAMe) occurred nearly 60 years ago (31). Widely known as the principal biological methyl donor, recent evidence illustrates its essential role in diverse cellular processes including growth and death. Although AdoMet is synthesized in all mammalian cells, liver can be considered as the body's AdoMet factory as this is where ~85% of all transmethylation reactions and 50% of methionine metabolism take place (67, 179). This review is focused mainly on the role of AdoMet in liver health, injury, and cancer. Much of current understanding has been gained through the use of genetic models that exhibit chronic hepatic AdoMet deficiency or excess. AdoMet is also available as a drug in many parts of the world and in the United States as a nutritional supplement. This review will also briefly discuss the current status of AdoMet's therapeutic use in liver diseases and several extrahepatic disorders.

Address for reprint requests and other correspondence: S. C. Lu, Keck School of Medicine, USC, HMR 415, 2011 Zonal Ave., Los Angeles, CA 90033 (shellylu@usc.edu)..

DISCLOSURES No conflicts of interest, financial or otherwise, are declared by the authors.

II. STRUCTURE AND BIOSYNTHESIS OF AdoMet

A. Structure of AdoMet

Early in evolution AdoMet emerged as the foremost donor of methyl groups in methylation reactions. Specific amino acid side chains in proteins, nucleotide bases in DNA and RNA, sugars, and many small molecules often carry a methyl group that is derived from AdoMet. Nature's selection of AdoMet for this role is attributable to its unique chemical structure. AdoMet is a typical sulfonium salt, a compound in which the sulfur atom has three single covalent substituents and therefore has a positive charge analogous to an ammonium compound (FIGURE 1). Structurally, sulfonium salts form a pyramidal arrangement of the three substituents, and there is a lone electron pair also associated with the sulfur atom (17). The sulfonium ion of AdoMet is particularly well suited for the transfer of its methyl group to a variety of nucleophilic atoms by a typical *sn*-2 substitution reaction with the concomitant release of *S*-adenosyl-L-homocysteine (AdoHcy) (FIGURE 1) (87, 229, 263). In AdoMet-dependent methylation reactions, the nucleophile may be a carbon atom, as in the *C*-methylation reaction of the C5-cytosine in DNA methylation; a nitrogen atom, as in the *N*-methylation reaction of the amino group in glycine methylation; an oxygen atom, as in the *O*-methylation reaction of the hydroxyl moiety in catecholamine methylation; or a sulfur atom, as in the *S*-methylation reaction of the sulfur atom in thiopurines methylation. AdoMet can also methylate other atoms, such as arsenic, an important step in the metabolism of this toxic metalloid (135).

Giulio Cantoni discovered AdoMet in 1953 (31). He elegantly demonstrated that methionine and ATP reacted to form a molecule, and after determination of its chemical structure, he named it AdoMet, capable of transferring a methyl group to nicotinamide or guanidinoacetic acid to form *N*-methylnicotinamide and creatine, respectively. Since then, investigators have implicated AdoMet in thousands of different methylation reactions in biological systems. In fact, AdoMet competes with ATP as the most widely used enzyme substrate (31, 138).

In addition to the chiral carbon at the α amino position, the sulfur at the sulfonium center of AdoMet is also chiral. Consequently, AdoMet can exist in two diastereomeric forms with respect to its sulfonium ion, (*S,S*)-AdoMet and (*R,S*)-AdoMet, where the designations refer to the sulfur and the α carbon, respectively. (*S,S*)-AdoMet is the only form that is synthesized enzymatically, whereas the AdoMet obtained by chemical methylation of AdoHcy is a racemic mixture of the two isomers (50). The (*S,S*) diastereomer is also the only form used in almost all methylation reactions (16, 17, 29, 56). Although under physiological conditions, 37°C and pH 7.5, the sulfonium ion of (*S,S*)-AdoMet can spontaneously racemize to the *R* form producing (*R,S*)-AdoMet with a first-order rate constant of 1.8×10^{-6} s^{-1} (91), the in vivo concentration of this biologically inactive AdoMet form is low to undetectable in the several tissues where it has been determined. Thus the (*R,S*)-AdoMet form was found to be only 1.5 and 3% of total AdoMet in mouse liver and rat brain, respectively (16, 91). This contrasts with the values predicted from the estimated racemization rate (91), which implies that in vivo there are mechanisms that either dispose of the (*R,S*)-AdoMet diastereomer or convert it back to (*S,S*)-AdoMet.

Another two spontaneous AdoMet reactions that take place under physiological conditions are: an intramolecular cleavage reaction that produces homoserine lactone and 5′-methylthioadenosine (MTA) (FIGURE 1) and a hydrolysis reaction that generates adenine and *S*-pentosylmethionine, which occur with first-order rate constants of 4.6×10^{-6} s^{-1} and 3×10^{-6} s^{-1}, respectively (91, 265). These two reactions may contribute to balance the racemization of (*S,S*)-AdoMet in vivo and prevent the accumulation of (*R,S*)-AdoMet. Two methyltransferases (MT), Mht1 (*S*-methylmethionine-homocysteine methyltransferase) and Sam4 (AdoMet-homocysteine methyltransferase), which transfer methyl groups from

AdoMet to homocysteine to synthesize methionine, have been identified in yeast, worms, plants, and flies (255). Sam4 was found to use both the (*S*,*S*) and (*R*,*S*) forms of AdoMet, with a higher affinity for the (*R*,S) diastereomer, while Mht1 was found to use exclusively the (*R*,*S*) form. *Mht1* and *Sam4* deletion in yeast resulted in accumulation of the biologically inactive (*R*,*S*) form, indicating that these two enzymes play an important role in controlling the in vivo accumulation of spontaneously generated (*R*,*S*)-AdoMet (256). Interestingly, Mht1 and Sam4 activities have not been detected in mammalian cells (257), which indicates that mammals limit (*R*,*S*)-AdoMet accumulation using other mechanisms.

It is important to note that spontaneous cleavage, hydrolysis, and racemization of AdoMet also take place in test tubes and tissue culture plates and that this may affect the interpretation of experimental results. For instance, MTA, a product of the spontaneous cleavage of AdoMet, in addition to being metabolized in vivo to form methionine can also inhibit certain MTs (54) (see sect. III*C*). When utilizing AdoMet, it is also important to note that racemization is independent of the pH in a range from 7.5 to 1.5, that the cleavage reaction persists up to a pH of 1.5, and that hydrolysis ceases at pH 6 (91). Finally, the chiral and chemical purity of AdoMet may vary between different commercial sources, which may affect its biological activity when used in the laboratory, as a food supplement, or in clinical settings.

B. Biosynthesis of AdoMet

Methionine adenosyltransferase (MAT) is the enzyme that catalyzes the biosynthesis of AdoMet from ATP and methionine, an essential amino acid (FIGURE 2). All living cells, with the only exception of some parasites and infectious agents that obtain AdoMet from their hosts (170, 236) (see sect. IX), express MAT. *MAT* is one of the genes that is essential to sustain life (80). Although divergent sequences have been reported for *Archea*, MAT has been extremely well conserved through evolution, with 59% sequence identity being found between the human and *Escherichia coli* enzymes (81). In mammals, all cells and tissues that have been studied express MAT, its activity being highest in the liver (67). Consequently, individuals with hepatic MAT activity deficiency are characterized by isolated persistent hypermethioninemia (248). The plasma methionine concentration in these subjects with hepatic MAT activity deficiency can be as high as 1,300 μM, while the upper limit in control individuals is ~35 μM (248).

The mechanism of AdoMet biosynthesis has been best studied using MAT purified from *Escherichia coli* and rat liver (58, 151). MAT activity is the combination of two consecutive reactions, AdoMet synthesis and hydrolysis of tripolyphosphate, with the latter being rate-limiting. The first reaction involves the formation of an enzyme-ATP-methionine complex and the synthesis of AdoMet and tripolyphosphate. In the second reaction, tripolyphosphate is hydrolyzed to orthophosphate and pyrophosphate before the release of the products. Tripolyphosphate is tightly bound to the enzyme so that its hydrolysis is oriented and over 95% of the orthophosphate generated originates from the γ-phosphoryl group of ATP. MAT is also able to hydrolyze exogenously added tripolyphosphate.

Ethionine is a nonproteinogenic amino acid structurally related to methionine with an ethyl group in place of a methyl group attached to the sulfur atom. Ethionine is a substrate of MAT and as such can react with ATP to form *S*-adenosylethionine, a nonmetabolizable analog of AdoMet (140). In vivo, ethionine causes hepatic AdoMet and ATP depletion, impaired methylation reactions, reduction of glucose and glycogen, lipid accumulation, and drug-induced liver injury (234). Cycloleucine, a nonmetabolizable, nonsubstrate analog of methionine synthesized by cyclization of leucine, is an inhibitor of MAT activity (140) that induces AdoMet depletion and potentiates drug-induced cytochrome *P*-450 2E1 (CYP2E1)-mediated oxidative stress and toxicity in hepatocytes (279). Nitric oxide (NO) reversibly

inhibits liver MAT tripolyphosphatase activity and also induces hepatic AdoMet depletion (58, 203, 222). This inhibitory effect of NO on MAT activity seems to be important for the in vivo regulation of AdoMet concentration during liver regeneration (253; see sect. IV*A*).

III. METABOLISM OF AdoMet

A. Transmethylation

AdoMet is the principal biological methyl donor made in the cytosol of every mammalian cell, but the liver is where the bulk of AdoMet is generated as it is the site where up to half of the daily intake of methionine is metabolized and up to 85% of all methylation reactions takes place (164, 179). AdoMet is the link to four key metabolic pathways: transmethylation (sect. III*A*), transsulfuration (sect. III*B*), polyamine synthesis (sect. III*C*), and 5′-deoxyadenosyl 5′-radical mediated biochemical transformations (sect. III-*D*). In transmethylation (FIGURE 2), AdoMet donates its methyl group to a large variety of acceptor molecules in reactions catalyzed by MTs. Over 200 proteins in the human genome have been identified as known or putative AdoMet-dependent MTs (204). These enzymes transfer a methyl group from AdoMet to a variety of nucleophiles, including oxygen, sulfur, nitrogen, and carbon atoms on proteins, nucleic acids, carbohydrates, lipids, and small molecules. The most abundant AdoMet-dependent MT in liver is glycine *N*-methyltransferase (GNMT), where it accounts for 1% of cytosolic protein (147, 237). Genetic deficiency of GNMT activity in humans has been reported in only three cases (6, 178). Metabolic abnormalities in these patients include elevated plasma methionine and AdoMet, which is consistent with this enzyme being the most abundant MT in liver (6, 178). Patients with GNMT deficiency develop hepatomegaly and liver injury at an early age (6, 178). Similarly, *Gnmt* knockout (KO) mice spontaneously develop steatohepatitis and HCC (see sect. VIII).

AdoMet-dependent methylation reactions yield AdoHcy as a byproduct. AdoHcy is a potent inhibitor of methylation reactions with an inhibition constant (K_i) that is in the same micromolar range as the K_m of most AdoMet-dependent MTs (45, 46). The ratio of AdoMet to AdoHcy is frequently considered as a metabolic gauge controlling in vivo methylation reactions, where a decrease in this ratio predicts reduced methylation capacity. However, it is important to note that since the activity of a given MT depends of its kinetic constants (mainly the K_m and K_i for AdoMet and AdoHcy, respectively), the methylation capacity is better determined by measuring the concentration of AdoMet and AdoHcy (69).

AdoHcy cellular content is regulated by the enzyme AdoHcy hydrolase (AHCY, also known as adenosylhomocysteinase), which reversibly cleaves this molecule into adenosine and homocysteine (Hcy) (FIGURE 2) (218). The thermodynamics of AHCY favor AdoHcy biosynthesis rather than hydrolysis (67). In vivo, the reaction proceeds in the direction of hydrolysis only if the products, adenosine and Hcy, are rapidly removed (90, 162), which is essential to prevent accumulation of AdoHcy.

Genetic deficiency of AHCY activity in humans has been reported in only a few cases (11, 12, 21, 82, 258). Metabolic abnormalities in all patients with AHCY deficiency include elevated plasma AdoHcy, AdoMet, and methionine, which confirms the important role of this enzyme in methionine metabolism. Plasma AdoMet and methionine accumulation in AHCY deficiency can be easily explained as the consequence of the inhibition of transmethylation reactions by AdoHcy and, therefore, of AdoMet utilization. Loss of function of the gene encoding AHCY in zebrafish larvae also induces an elevation of the content of AdoHcy and AdoMet with a reduction of the AdoMet-to-AdoHcy ratio (167). AHCY deficiency in zebrafish induced a decrease in global DNA methylation and global

protein lysine methylation, which is consistent with a global inhibition of MTs caused by elevated AdoHcy and decreased AdoMet-to-AdoHcy ratio.

Whereas the patients with the mildest clinical symptoms have 20–30% residual AHCY activity and develop myopathy, delayed development, steatosis and abnormal liver function tests, patients with very low or undetectable resid ual AHCY activity have fetal hydrops (probably due to impaired liver function), severe hypotonia/myopathy, feeding problems, respiratory failure, and develop tumors and die soon after birth (11, 12, 21, 82, 258). Since the two published cases of fetal hydrops associated with AHCY deficiency are two siblings (82), it is not possible to exclude that this condition is not due to some other family trait independent of the AHCY deficiency. Hepatic steatosis also develops spontaneously in zebrafish deficient in AHCY activity (167).

AHCY is a reversible enzyme that favors AdoHcy biosynthesis so that impairment in Hcy catabolism will lead to AdoHcy accumulation and inhibition of MTs. In liver, Hcy can undergo remethylation or conversion to cysteine via the transsulfuration pathway. Remethylation of Hcy to form methionine occurs by two enzymes: methionine synthase (MS), which requires normal levels of folate and vitamin B_{12}, and betaine Hcy methyltransferase (BHMT), which requires betaine, a metabolite of choline (FIGURE 2). MS-catalyzed Hcy remethylation requires 5-methyltetrahydrofolate (5-MTHF), which is derived from 5,10-methylenetetrahydrofolate (5,10-MTHF) in a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR). 5-MTHF is then converted to tetrahydrofolate (THF) as it donates its methyl group and THF is converted to 5,10-MTHF to complete the folate cycle.

B. Transsulfuration

The transsulfuration pathway (FIGURE 3*A*) links AdoMet to cysteine biosynthesis. Here Hcy is converted to cysteine (the rate-limiting precursor for GSH synthesis) via a two-step enzymatic process catalyzed by cystathionine β-synthase (CBS) and cystathionase (CSE), both requiring vitamin B_6. The transsulfuration pathway is particularly active in the liver, making AdoMet an important precursor of GSH (142). All mammalian tissues express MAT and MS, whereas BHMT is limited to the liver and kidney. In the liver, AdoMet inhibits MTHFR (therefore reduces 5-MTHF, the substrate for MS) and activates CBS (162, 207). This way AdoMet level controls the flux so that when it is depleted, Hcy is channeled to remethylation to regenerate AdoMet, whereas when the level is high, Hcy is channeled to the transsulfuration pathway. Accordingly, whereas mice deficient in BHMT or MTHFR have increased levels of Hcy and AdoHcy as well as reduced AdoMet, CBS deficiency associates with elevated Hcy, AdoHcy, and AdoMet (68).

Although the classical role of CBS and CSE is to generate cystathionine and cysteine, respectively, these two enzymes catalyze multiple hydrogen sulfide (H_2S)-generating reactions using cysteine and homocysteine as substrates (FIGURE 3*B*) (104). Whereas H_2S production from cysteine is primarily catalyzed by CSE, H_2S generation by CBS occurs mainly by condensation of cysteine and homocysteine (104). A third pathway uses aspartate aminotransferase (AST), which also deaminates cysteine to yield mercaptopyruvate that is further converted into H_2S and pyruvate in a reaction catalyzed by mercaptopyruvate sulfurtransferase (MST) (FIGURE 3*B*) (104). Whereas CBS regulates H_2S production mainly in the brain, CSE is principally responsible for H_2S synthesis in the periphery (75). The high K_m of AST for cysteine is consistent with the function of this enzyme, together with MST, in the cysteine catabolic pathway (104).

H_2S is a signaling molecule that has been shown to reduce systemic blood pressure causing vasodilation of mesenteric artery, aorta, and portal vein (75). Decreased CSE expression

coupled with reduced plasma H_2S contributes to the development of portal hypertension (70), the main complication of cirrhotic liver. *Cse* KO mice develop normally, although they display hypercystationinemia, hyperhomocysteinemia, and reduced levels of serum H_2S (100, 269). Hypermethioninemia and hepatic dysfunction/steatosis, a characteristic feature of *Cbs* KO mice as well as of other mouse models displaying elevated plasma Hcy levels (166), are not observed in *Cse*-deficient animals (100, 269). These results indicate *1*) that peripheral H_2S does not play an important role in the initiation of liver injury, although it may be important in the development of portal hypertension in cirrhosis, and *2*) that hyperhomocysteinemia alone does not induce hepatic injury in mice.

C. Polyamine Biosynthesis

AdoMet is best known as the principal biological methyl donor. However, in addition to this well-recognized function, AdoMet is critically involved in the synthesis of polyamines. Polyamines are low-molecular-weight, positively charged molecules that are ubiquitous in all living cells (201). Polyamines play a crucial role in many biochemical processes including regulation of transcription and translation, cell growth, and apoptosis. To enter this pathway, AdoMet needs first to be decarboxylated, a reaction catalyzed by the enzyme AdoMet decarboxylase, which is the rate-limiting step for polyamine synthesis since decarboxylated AdoMet (dcAdoMet) content is low to not detectable in cells under normal conditions (FIGURE 4). The predominant polyamines in mammalian cells are spermidine (SPD) and spermine (SPM). These polyamines are made by sequential addition of aminopropyl groups from dcAdoMet, yielding MTA as a byproduct. SPD synthase catalyzes the transfer of the first aminopropyl group from dcAdoMet to putrescine to form SPD and MTA, whereas the addition of the second aminopropyl group to the *n*-10 position of SPD to form SPM and a second molecule of MTA is catalyzed by SPM synthase.

MTA is a potent inhibitor of SPM synthase with a K_i of ~0.3 μM (89, 192, 193) and a less potent inhibitor of SPD synthase with a K_i of ~2–10 μM (89, 210). This inhibition, however, does not have a great impact on polyamine synthesis in vivo since MTA is rapidly metabolized, through the methionine salvation pathway, to regenerate AdoMet (FIGURE 4) (200). The first reaction in this pathway is the phosphorylation of MTA by the enzyme MTA phosphorylase (MTAP). MTAP cleaves MTA to adenine and 5-methylthioribose-1-phosphate, and the latter compound is further metabolized to methionine. Cells lacking MTAP cannot salvage methionine or adenine from endogenous MTA, which leads to impaired polyamine biosynthesis and to the accumulation of dcAdoMet and MTA. dcAdoMet is an inhibitor of methylation reactions, such as DNA methylation (73, 86), as this compound cannot donate its methyl group. MTA is both an inhibitor of methylation reactions (54, 119, 264) and an inhibitor of AHCY (208). AHCY, as mentioned in section III*A*, is a reversible enzyme that cleaves AdoHcy, a potent inhibitor of methylation reactions, into adenosine and Hcy. Thus MTAP deficiency not only leads to an impaired biosynthesis of polyamines, but also to the inhibition of methylation reactions caused by the cellular accumulation of dcAdoMet, MTA, and AdoHcy.

MTAP has been proposed to be a tumor suppressor gene, and as such, downregulation of MTAP has been associated with tumor progression both in experimental animal models and in humans (18). Loss of *Mtap* in mice induces embryonic lethality, but heterozygous *Mtap* KO mice are viable and although indistinguishable from wild-type animals, tend to die prematurely of T-cell lymphoma (103). How MTAP-deficient tumor cells escape from the deleterious effects of an excessive accumulation of MTA is not clear. Changes in AdoMet synthesis (25) and MTA excretion (111) as well as changes in the polyamine biosynthetic and catabolic pathways (108, 109, 199) may be among the mechanisms used by cancer cells to adapt to a deficiency in MTAP activity. Interestingly, MTA has been shown to be pro-apoptotic in Huh-7 and HepG2, two hepatoma cell lines, but anti-apoptotic in normal

hepatocytes (4, 9, 275). Similarly, MTA has been shown to be pro-apoptotic in colon cancer cells but not in normal colon epithelial cells (131), and MTA has also been found to reduce the growth of preneoplastic lesions in rat liver during the early stages of hepatocarcinogenesis (197, 198).

Inhibition of residual MTAP with picomolar methylthio-DADMe-immuncillin-A (MTDIA) increases cellular MTA concentration, decreases polyamine synthesis and DNA methylation, and induces apoptosis in FaDu and Cal27, two head and neck squamous cell carcinoma cell lines, but not in normal human cell fibroblasts or in MCF7, a breast cancer cell line where *MTAP* is deleted (14). Furthermore, inhibition of MTAP with MTDIA produces an increase in the intracellular MTA concentration and blocks A549 (human non-small cell lung carcinoma cells) and H358 (human bronchioalveolar non-small cell lung carcinoma) xenograft tumor growth in immune-deficient nude mice (15).

These findings, along with those made in cells and animal models defective in AdoMet biosynthetic and catabolic pathways (see sects. VII and VIII), indicate that metabolic fluxes through the transmethylation and the polyamine pathways and the consequent changes in levels of various metabolites within the cell (i.e., AdoMet, dcAdoMet, MTA, AdoHcy, and methionine) are critical in tumor development, and that alteration of this onco-metabolic homeostasis, as exemplified by treatment with MTA, can block cell growth and induce apoptosis.

D. AdoMet Radical Reactions

In 1976, Knappe and Schmitt (112) elegantly demonstrated that apart from its well-known function as a methyl donor and precursor of polyamine synthesis, AdoMet also mediates novel radical chemical reactions in organisms grown anaerobically (112). This family of AdoMet radical enzymes currently comprises more than 2,800 members that share a CX_3CX_2C motif forming a characteristic [4Fe–4S] cluster (72). This [4Fe–4S] cluster binds AdoMet to the only Fe not bound to a cysteine residue catalyzing its reductive cleavage to generate [4Fe–4S]-methionine and a 5′-deoxyadenosyl 5′-radical (FIGURE 4). 5′-Deoxyadenosyl 5′-radical then removes a hydrogen atom from a small molecule, protein, RNA, or DNA substrate to initiate a radical mechanism. AdoMet radical enzymes participate in more than 40 distinct biochemical transformations, and most members have not been biochemically characterized (72). Viperin, an interferon (IFN)-inducible antiviral protein (228), has been recently demonstrated to be an AdoMet radical enzyme, although the substrate for this reaction has not been identified (61). Viperin is one of the IFN-stimulated genes whose expression in liver biopsy specimens of chronic hepatitis C patients is predictive of the response to pegylated-IFN-α/ribavirin treatment (228). Interestingly, AdoMet improves early viral responses and IFN-stimulated gene induction in chronic hepatitis C patients (63, 66), whether AdoMet radical reactions are mediating this effect of AdoMet is unknown.

IV. AdoMet's EFFECT ON GROWTH AND APOPTOSIS

A. AdoMet's Effect on Growth

Accumulating evidence shows that AdoMet regulates cellular proliferation. It is important to point out that the effect is cell-type specific. Thus intracellular AdoMet level increases nearly fivefold during lymphocyte activation, and this increase appears to be essential for activation presumably by providing a source for polyamines (83). This is not the case in hepatocytes, where AdoMet level is related to the differentiation status so that quiescent and proliferating hepatocytes have high and low AdoMet levels, respectively (25, 155). Hepatic AdoMet level is dramatically reduced shortly after two-thirds partial hepatectomy (PH), coinciding with the onset of DNA synthesis and the induction of early response genes (96).

Hepatocyte DNA synthesis was inhibited when this fall in AdoMet was prevented by exogenous AdoMet administration (198, 253). This is further illustrated by the *Mat1a* KO mouse model, which exhibits increased basal hepatocyte proliferation but impaired regeneration following PH (42). In this model, the basal hepatic AdoMet level is reduced but did not change following PH (42). Impaired liver regeneration is also observed in *Gnmt* KO mice, where hepatic AdoMet levels are markedly elevated (251).

Several molecular mechanisms of AdoMet's growth inhibitory effect in hepatocytes have been elucidated. One important mechanism is the ability of AdoMet to inhibit mitogenic activity of growth factors. Examples include the hepatocyte growth factor (HGF), the most potent hepatocyte mitogen essential for liver regeneration (122), and leptin (214). Both growth factors activate *MAT2A* transcriptionally and induce hepatocyte DNA synthesis (122, 214). *MAT2A* induction has been shown to be required for liver cell proliferation (194, 214). AdoMet prevented *MAT2A* induction and inhibited the mitogenic activity of HGF and leptin (122, 214). Although HGF is a potent mitogen in vitro, it elicits a poor mitogenic response when administered in vivo to normal rats (173). These observations suggest that hepatic parenchymal cells need to be primed to respond to proliferative signals. Two key inflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-6, are part of this priming event (52, 268). Both cytokines promote the activation of signaling pathways including NFκB, STAT-3, activator protein 1 (AP-1), and CCAAT/enhancer binding protein β (C/EBP β) that lead to activation of target genes in hepatocytes leading to DNA synthesis and cell proliferation (250). One target gene induced is inducible NO synthase (iNOS), which also occurs before the onset of DNA synthesis (185). Increased NO production is essential for liver regeneration as mice lacking iNOS have impaired liver regeneration (209). NO is known to inactivate MAT I/III (but not MAT II) (see sect. V*A*), and interestingly, the mitogenic activity of HGF in cultured hepatocytes required iNOS activity and a fall in AdoMet level (77). These observations suggest a scenario where the influx of inflammatory cytokines following PH induces iNOS expression and increases NO production, leading to inactivation of MAT I/III and a fall in AdoMet level. This then releases the inhibitory tone that AdoMet exerts on growth factors. Consistent with this, *Mat1a* KO mice do not exhibit a fall in AdoMet level following PH, and *Mat1a* KO hepatocytes are resistant to the mitogenic effect of HGF (42).

Recent work has shed further insight on the HGF-NO link in hepatocyte proliferation. HGF activates the Met receptor tyrosine kinase signaling cascade, leading to the activation of the Ras/ERK/MAPK, phosphatidylinositol 3-kinase (PI3K)/AKT, Rac/Pak (serine/threonine kinase p21-activated kinase), and Crk/Rap1 pathways (20). Recently, a noncanonical signaling pathway was identified for HGF involving serine/threonine kinase 11 (LKB1)/AMP-activated protein kinase (AMPK) that is essential for hepatocyte proliferation (253). LKB1 lies upstream of AMPK, and both are considered commonly as metabolic tumor suppressors (231). However, in hepatocytes, HGF treatment activates LKB1/AMPK signaling, leading to nuclear to cytosolic translocation of the RNA-binding protein HuR, which in turn increases the half-life of target mRNA such as cyclin D1 and A2 to promote hepatocyte proliferation (159, 253). AdoMet can block HGF-mediated activation of LKB1/AMPK signaling by a mechanism that requires protein phosphatase 2A (PP2A) (159), presumably by increasing the level of methylated PP2A (mePP2A), which is known to have higher activity (40, 127). The link between HGF's mitogenic activity and NO is through AMPK, which can phosphorylate and activate endothelial NO synthase (eNOS) (128), and mice lacking eNOS have impaired liver regeneration (253). Taken together, the working scheme is summarized in FIGURE 5 as follows: *1*) HGF induces NO production through the activation of eNOS phosphorylation, via LKB1 and AMPK activation, and the eNOS derived NO contributes to the stimulation of NO synthesis via iNOS; *2*) this increase in hepatic NO inactivates MATI/III, leading to a fall in AdoMet level and reduction in the

mePP2A/demethylatedPP2A ratio; and *3*) this reduction in PP2A activity facilitates LKB1/AMPK phosphorylation, cell cycle progression, and growth.

In the case of leptin, AdoMet was able to block its mitogenic effect in HepG2 cells by preventing leptin-mediated activation of ERK and AKT (214). Interestingly, AdoMet was also shown to block transforming growth factor-β (TGF-β)-mediated ERK activation in hepatic stellate cells (HSCs), and this may be one of its antifibrogenic effects (181). In addition to the liver, AdoMet also blocks the mitogenic effects of leptin, epidermal growth factor (EGF), and insulin-like growth factor (IGF)-I in colon cancer cell lines (41). How AdoMet blocks the effect of these mitogens on ERK, AKT signaling remains largely unknown.

The antiproliferative effect of AdoMet can be recapitulated by MTA (41, 77, 214). As discussed above, MTA is a key metabolite of AdoMet generated in the polyamine pathway as well as a product of AdoMet via nonenzymatic breakdown. In contrast to AdoMet, MTA is not a methyl donor and inhibits polyamine synthesis and MTs by multiple mechanisms (48, 54). While AdoMet is highly unstable with a short half-life (12 h at 37°C in culture medium), MTA is very stable (41). The rate of conversion from AdoMet to MTA is 0.013 mM per hour per mM AdoMet under the same condition (41). These observations also suggest that many of AdoMet's biological effects, when delivered at pharmacological doses, could be mediated in part through its conversion to MTA.

B. AdoMet's Effect on Apoptosis

AdoMet also regulates hepatocyte's death response. AdoMet is known to ameliorate many liver injuries where apoptosis plays a contributory role (see sect. IX). Part of its beneficial effect is due to the fact that in normal hepatocytes, AdoMet is antiapoptotic (4). The protection may be partly related to raising the antioxidant capacity as a GSH precursor and suppressing TNF-α induction (98, 261). However, while AdoMet protected against okadaic acid-induced apoptosis in normal hepatocytes, it induced apoptosis in liver cancer cell lines HepG2 and Huh-7 (4). MTA also recapitulated these actions. These findings are consistent with the chemopreventive action of AdoMet and MTA in an in vivo model of hepatocarcinogenesis in rats, which was accompanied by increased apoptotic bodies in atypical nodules and HCC foci in AdoMet-treated animals (196).

One mechanism of AdoMet and MTA's differential effect on apoptosis in normal hepatocytes and liver cancer cells is due to their ability to selectively induce Bcl-x_S in liver cancer cells but not normal hepatocytes (275). Bcl-x_S and Bcl-x_L are splice variants of Bcl-x; Bcl-x_L is anti-apoptotic, while Bcl-x_S is pro-apoptotic (19). Alternative splicing requires dephosphorylation of SR proteins (176), which are specific substrates of protein phosphatase 1 (PP1) (36). Both AdoMet and MTA increased PP1 catalytic subunit mRNA and protein levels and dephosphorylation of SR proteins, leading to increased alternative splicing of Bcl-x (275). This also points out that AdoMet and MTA can affect cellular phosphorylation state in liver cancer cells. AdoMet and MTA also have a selective proapoptotic activity against colon cancer cell lines but not normal colonic epithelial cells (131). However, the mechanism is different from in liver cancer cells, as Bcl-x_S expression was not affected. Instead, these molecules inhibited the expression of cellular FLICE inhibitory protein (cFLIP), resulting in activation of procaspase-8, Bid cleavage, and release of cytochrome *c* from the mitochondria (131). This further illustrates that the effects of these molecules are cell-type specific.

V. METHIONINE ADENOSYLTRANSFERASE GENES AND ISOENZYMES

A. MAT Genes, Isoenzymes, Kinetics, and Posttranslational Regulation

Mammals express two genes, *MAT1A* and *MAT2A*, which encode for two homologous MAT catalytic subunits, α1 and α2 (118). *MAT1A* is mostly expressed in normal liver, and the α1 subunit organizes into two MAT isoenzymes, MAT III (dimer) and MAT I (tetramer) (118). *MAT2A* is widely distributed, and it encodes for a catalytic subunit (α2) found in the MAT isoenzyme MAT II that also exists in polymeric forms that vary from tissue to tissue (92, 117). Mammals also express a third gene, *MAT2B*, which encodes for a β-regulatory subunit that regulates the activity of MAT II by lowering the K_i for AdoMet and the K_m for methionine (83, 125). Recently we reported that *MAT2B* encodes for two dominant splicing variants, V1 and V2, and V1 is the β-regulatory subunit but V2 differs from V1 by 20 amino acids in the NH_2 terminus (272). *MAT2B* is expressed in most but not all tissues, and the two variants are differentially expressed in normal tissues; prostate, lung, thyroid, adrenal gland, fetal liver, and brain express mostly V1, whereas skeletal muscle and heart express mostly V2. Thymus and kidney express both variants (272). In addition to interacting with MAT II, both MAT2B variants can interact with many other proteins, including HuR (267), and participate in the regulation of growth and apoptosis (155, 272) by mechanisms that appear to be independent of AdoMet.

MAT isoenzymes differ in kinetic and regulatory properties and sensitivities to inhibitors of MAT (146). MAT III has highest (215 μM to 7 mM), MAT I has intermediate (23 μM to 1 mM), and MAT II has the lowest (~4–10 μM) K_m for methionine (146). AdoMet strongly inhibits MAT II (IC_{50} = 60 μM), whereas it minimally inhibits MAT I (IC_{50} = 400 μM) and stimulates MAT III (up to 8-fold at 500 μM AdoMet) (238). The K_m for ATP is also highest for MAT III (1–2 mM), intermediate for MAT I (0.2–0.5 mM), and lowest for MAT II (70 μM) (186, 190). Since the intracellular methionine concentration in hepatocytes is in the range of 50–80 μM (67), MAT II and MAT I would be the predominantly active MAT under physiological conditions, assuming equal amounts of isoenzymes are present. Given the difference in kinetics and regulatory properties, the type of MAT isoenzyme expressed can influence cellular AdoMet levels. Consistent with this, cells that express *MAT1A* have much higher steady-state AdoMet levels than cells that express *MAT2A* (25). Cells that express only MAT II have AdoMet levels that should be relatively unaffected by fluctuations in methionine availability because of negative feedback inhibition. However, expression of *MAT2B* can influence this as the β regulatory subunit renders MAT II more susceptible to feedback inhibition by AdoMet (83). Indeed, during lymphocyte activation, the β-subunit disappears, which may explain the nearly fivefold increase in AdoMet levels compared with resting cells (83). In liver cancer cells, overexpression of *MAT2B* lowered (155), while knockdown of *MAT2B* raised AdoMet content (214). In contrast, in cells that express only MAT1A, AdoMet level increases with increasing methionine availability (67), which agrees with the observation that both methionine and AdoMet activate MAT III (24, 58).

MAT isoenzymes differ in responsiveness to sulfhydryl modifying agents. This is because the cysteine at position 121, conserved in rat and human MAT α1 but absent in α2, is a target of covalent modification (10, 203, 222, 226). Based on the model structure of the rat MAT I/III, cysteine 121 is localized at a “flexible loop” over the active site cleft of MAT (203, 226). Although this cysteine is not essential for activity, when cysteine is modified either by oxidation or nitrosylation, the enzyme is inactivated (10, 174, 203, 222, 226). GSH and other thiol-reducing agents can reverse this inactivation. MAT III (dimer) requires 3 mM while MAT I (tetramer) requires 25 mM GSH to reverse the inactivation (226). Since normal hepatic GSH concentration is 5–10 mM, the difference may also contribute to the selective loss of the tetramer in liver disease (23, 51). MAT I/III nitrosylation and

inactivation have been demonstrated both in vitro and in vivo in animals treated with LPS (10, 222). MAT I/III have been shown to be phosphorylated by PKC (191), but it did not alter the overall kinetic parameters and is unclear whether phosphorylation plays any physiological role in the regulation of the enzyme activity. Whether MAT II is regulated by phosphorylation is unknown.

In addition to catalyzing the synthesis of AdoMet in cytosol, recent work from Reytor et al. (217) showed that MAT I/III exhibit two partially overlapping areas at its COOH-terminal end that are involved in cytoplasmic retention and nuclear localization. Both tetrameric and monomeric forms of α1 are localized to the nucleus. The authors speculate that the presence of nuclear MAT might be involved in providing a continuous source of nuclear AdoMet. In support of this, nuclear accumulation of the active MAT1A protein correlated with higher levels of histone H3K27 trimethylation, an epigenetic modification associated with gene repression and DNA methylation (217). We reported that MAT2B variants are largely localized to the nucleus and interact with many proteins including HuR, the RNA binding protein known to stabilize many mRNAs (267). Recently, MAT II has also been reported to be mostly nuclear where it serves in conjunction with the β-subunit as a transcriptional corepressor complex of MafK (107). These investigators found that MAT II interacts with many nuclear proteins and may provide a source of AdoMet for methylation of histones or DNA to participate in chromatin-based regulations (107). This accumulating evidence suggests the products of *MAT* genes are involved in regulation of many pathways, some of which are chromatin-based and some may be independent of AdoMet.

B. MAT Gene Regulation and Dysregulation

Fetal liver expresses *MAT2A* and *MAT2B* but not *MAT1A* (78, 272). *MAT1A* expression increases a few days after birth and progressively takes over so that the adult liver (mostly hepatocytes) expresses mainly *MAT1A*, very little *MAT2A*, or *MAT2B* (78, 272). Thus *MAT1A* can be considered a marker of differentiated liver phenotype (164). *MAT1A* expression is stimulated by glucocorticoid and cAMP (47, 78, 225, 277). Glucocorticoid also induced human *MAT1A* promoter activity and expression (277). Chronic hypoxia decreased rat *MAT1A* gene expression (8, 38). Whether the same occurs for the human gene is unknown. In the rat, TNF-α was shown to downregulate *MAT1A* expression, an action mediated by acidic sphingomyelinase and was achieved via accelerated mRNA degradation (150). Recently, thyroid hormone T_3 was shown to positively regulate human *MAT1A* indirectly via C/EBP α and β at the transcriptional level (266). Several putative C/EBP binding sites are present in the human and murine *MAT1A* promoter region, and although the human gene is regulated by both C/EBPα and β, only C/EBPβ has been shown to positively regulate the murine gene (99, 266). Lastly, sterol regulatory element-binding protein 1a (SREBP1a), a transcription factor that regulates transcription of many genes involved in fatty acid biosynthesis, was recently identified to positively regulate *MAT1A* at the mRNA level, although the precise mechanism is unclear (259).

Hepatic *MAT1A* gene expression is downregulated in HCC (26), during dedifferentiation (146, 254), in most cirrhotic patients (7), and in patients with alcoholic hepatitis (123). The mechanisms of *MAT1A* downregulation occur both at transcriptional and posttranscriptional levels. The rat and murine *MAT1A* expression is regulated by epigenetics, namely, promoter methylation and histone acetylation (99, 245). Elevated levels of histone acetylation help to maintain a decondensed and active state of the chromatin, and the underlying pattern of CpG methylation modulates histone acetylation (44, 233). Indeed, the degree of acetylation of histones (H4) associated with the rat *MAT1A* promoter in the liver is ~15-fold higher than in the kidney (245). The human *MAT1A* gene is similarly regulated as its expression can be induced by treatment with a demethylating agent or inhibitor of histone deacetylase in HepG2 cells (245). Furthermore, in HepG2 cells and human cirrhotic livers, decreased

MAT1A expression is associated with hypermethylation of a *Hpa*II site at −977 position (numbered relative to the translational start site) of the human *MAT1A* promoter (7, 245). However, more recent examination showed that methylation at +10 and +88, but not −977, correlated better with lack of human *MAT1A* expression in HCC (242). *MAT1A* promoter construct methylated at +10 or +88, but not −977, exhibited reduced reporter activity. This was due to reduced transcription. Thus, instead of promoter hypermethylation, it is coding region methylation close to the translational start site of human *MAT1A* that can interfere with gene transcription (242).

One mechanism of posttranscriptional regulation of human *MAT1A* involves binding of the AU-rich RNA binding factor 1 (AUF1) to the *MAT1A* 3′-UTR region (254). AUF1 is known to destabilize target mRNAs (278). HCC specimens express higher AUF1 protein levels, and knockdown of AUF1 increased *MAT1A* mRNA level (254). In addition, AUF1 expression is high in fetal liver and falls during liver development, which coincides with increased *MAT1A* expression (254).

While *MAT1A* is a marker for normal differentiated liver, *MAT2A* is a marker for rapid liver growth and dedifferentiation. *MAT2A* is transcriptionally induced in human HCC (26), during rapid liver growth, dedifferentiation, and in response to ethanol feeding in rodents (96, 97, 145). Like *MAT1A*, *MAT2A* is also regulated at transcriptional and posttranscriptional levels. We have identified four *cis*-acting elements and *trans*-activating factors (Sp1, c-Myb, NFκB, and AP-1) to be involved in *MAT2A* transcriptional upregulation in HCC (271, 276). TNF-α upregulates *MAT2A* via NFκB and AP-1 (276). During liver regeneration in rats, *MAT2A* induction occurs during hepatocyte priming (G_0-G_1 transition) and at the G_1-S interface, and Sp1 plays a critical role for *MAT2A* induction during the second phase (219). There is an adjacent E2F binding site in both human and rat *MAT2A* promoter, and cooperation between E2F and Sp1 occurs to induce the *MAT2A* expression (219). Promoter methylation and histone acetylation status also regulate *MAT2A* expression. Specifically, the human *MAT2A* promoter is hypomethylated in HCC but hypermethylated in normal liver (270). Histone acetylation status correlates with *MAT2A* expression in both human and rat so that a hyperacetylated status correlates with high *MAT2A* expression (122, 270). Indeed, HGF induces MAT2A expression in rat hepatocytes via stimulation of histone (H4) acetylation associated with the *MAT2A* promoter (122). Recently, Liu et al. (137) reported that hypoxia-inducible factor 1α (HIF-1α) can induce the human *MAT2A* promoter activity that was abolished when the putative HIF-1α binding motif was mutated, confirming its functionality (137). This may represent another mechanism of *MAT2A* induction in HCC as HIF-1α is often overex-pressed (137).

HuR and methylated HuR regulate *MAT2A* expression at the posttranscriptional level (254). While HuR is a ubiquitously expressed RNA binding protein known to stabilize its target mRNAs, methylated-HuR exerts the opposite effect (254). During hepatocyte dedifferentiation and in HCC, methylated-HuR level falls while HuR level increases, and there is a switch from methylated-HuR to HuR binding to the 3′-UTR of MAT2A, resulting in increased MAT2A mRNA level. The opposite occurs during fetal development, resulting in a fall in *MAT2A* expression (254).

Similar to *MAT2A*, *MAT2B* is also expressed minimally in normal adult liver (272). In human liver, *MAT2B* expression is increased in cirrhosis and HCC (155, 272). Of the two dominant MAT2B splicing variants, TNF-α induces V1 expression (but not V2) at the transcriptional level by mechanisms that involve AP-1 and NFκB (272). Leptin induces the expression of both *MAT2A* and *MAT2B* at the transcriptional level in human hepatoma cells by mechanisms that involve ERK and AKT signaling (214). This induction is essential for leptin to exert its mitogenic effect (214). Since leptin is a profibrogenic factor and

MAT2A and *MAT2B* are essential for growth, we also examined their expression during HSC activation in vitro and in vivo. Both genes are highly induced during HSC activation and are required for HSCs to be activated. During HSC activation, there is a fall in cellular AdoMet level and global DNA hypomethylation. *MAT2A* induction appears to be essential to provide a source of AdoMet for the growing cells, while *MAT2B* (not *MAT2A*) expression is required for ERK and PI3K signaling to occur so that blocking the expression of either *MAT2A* or *MAT2B* inhibited HSC activation (213). *MAT2B* expression is also required for leptin-mediated activation of ERK, PI3K, and STAT3 in hepatoma cells (214). The molecular mechanism(s) of how *MAT2B* regulates these signaling pathways remains to be elucidated. Whether *MAT2B* is regulated posttranscriptionally is unknown.

While *MAT* expression can influence the steady-state AdoMet level (25), AdoMet level can influence *MAT* expression in return. *MAT1A* expression falls while *MAT2A* is induced in primary cultures of hepatocytes due to dedifferentiation (76, 241). This change is prevented by the addition of AdoMet. The opposite occurs with *MAT2A* so that *MAT2A* gene expression is rapidly induced when AdoMet level falls (by restricting L-methionine in medium) and downregulated when AdoMet is added (156, 270). AdoMet treatment results in higher methylated-HuR level, which destabilizes the *MAT2A* mRNA and may explain its inhibitory effect on *MAT2A* expression (254). AdoMet can also inhibit *MAT2B* expression at baseline and prevent its induction by leptin in hepatoma cells (214). TABLE 1 summarizes properties and regulation of mammalian MATs.

VI. GLYCINE *N*-METHYLTRANSFERASE

After a protein-rich meal, serum methionine concentration increases transiently as it gets rapidly metabolized in the liver through the concerted action of MAT III and GNMT, which are allosterically activated by methionine and AdoMet, respectively (161). The products of the reaction catalyzed by GNMT are AdoHcy and sarcosine (FIGURE 6). Sarcosine has no known essential metabolic function and is demethylated by the mitochondrial flavoprotein sarcosine dehydrogenase (SDH) to regenerate glycine. During this reaction, THF bound to SDH is converted to 5,10-MTHF as a byproduct (FIGURE 6) (206). 5,10-MTHF is then used to regenerate methionine, via its conversion to 5-MTHF, an inhibitor of GNMT (FIGURE 2). Through this intricate set of reactions, the methyl group of AdoMet used by GNMT is not lost but is saved from being used in undesired methylation reactions of nucleic acids, proteins, etc., to be recovered, via the folate cycle, to regenerate methionine (see sect. III*A*). Together, these results indicate that the function of GNMT is to act as a cellular buffer that maintains constant the concentration of AdoMet.

Consistent with this concerted action of MAT III/GNMT to maintain AdoMet concentration within a tight range and to prevent aberrant methylation reactions, the tissue expression profile of *GNMT* parallels that of *MAT1A*. Thus GNMT is present in large amounts in the liver (1–3% cytosolic protein) and in pancreas and prostate (0.4% of cytosolic protein) and in smaller amounts in other tissues such as kidney tubules, submaxillary glands, intestinal mucosa, cortical neurons, and Purkinje and Schwann cells (149). Similar to *MAT1A*, the expression of *GNMT* is minimal in livers of embryos, increases rapidly after birth, and is low or undetectable in liver and prostate tumors; both *MAT1A* and *GNMT* expression are induced by glucocorticoids (79, 149). As it occurs with *MAT1A*, *GNMT* is also expressed in the nuclei of liver cells, as well as other cells, although in small amounts (149). The function of nuclear GNMT is still a matter of speculation, but a simple hypothesis is that the content of AdoMet in this organelle needs to be particularly well regulated to prevent aberrant epigenetic modifications.

In *Gnmt* KO mice, hepatic AdoMet concentration is increased ~40-fold, methionine is increased ~7-fold, AdoHcy content is reduced ~3-fold, and the AdoMet-to-AdoHcy ratio, which is an index of the liver methylating capacity, is increased ~100-fold (148, 158). Consistently there is global DNA hypermethylation and increased histone methylation (148, 158). These results confirm the hypothesis that the main biological function of GNMT is to regulate AdoMet content and thus control the AdoMet-to-AdoHcy ratio. In patients with GNMT deficiency and in *Gnmt* KO mice, serum methionine is also markedly increased (6, 158, 178). Because *MAT1A* expression is not affected in *Gnmt* KO mice (158) and MAT III has a large capacity to catabolize methionine and is activated by both methionine and AdoMet (see sect. V*A*), it is puzzling that *Gnmt* KO mice have increased serum and liver methionine levels. One possible explanation is that GNMT deficiency causes an increased flux of AdoMet through the methylation-independent pathways (polyamine biosynthesis and the AdoMet radical superfamily) leading to a rapid regeneration of methionine (see FIGURE 4 and sect. III, *C* and *D*). Since methionine is rapidly and reversibly transported through the hepatocytes (116), this may provide a mechanism to explain why serum and liver methionine levels are increased in *Gnmt* KO mice. Increased serum methionine may also result from the inhibition of MAT II in extrahepatic tissues caused by AdoMet effluxing from the liver. However, none of these hypotheses has been experimentally explored.

VII. CONSEQUENCES OF CHRONIC HEPATIC AdoMet DEFICIENCY

The *Mat1a* KO mouse model has provided much insight into the consequences of chronic hepatic AdoMet deficiency and altered signaling pathways that may lead to malignant degeneration. This model is highly relevant to human liver disease as *MAT1A* expression is markedly diminished in the majority of patients with cirrhosis (7). This is the result of reduced or absent *MAT1A* mRNA and inactivation of MAT I/III as a result of nitrosative and oxidative stress (164). Reduced hepatic MAT activity also explains why many cirrhotic patients have hypermethioninemia and delayed plasma clearance of methionine after intravenous injection (7, 94, 110, 223). Recently, methionine metabolism (whole body flux of methionine, its rate of transmethylation and transsulfuration) has been studied in patients with nonalcoholic steatohepatitis (NASH) after intravenous injection of trace amounts of L-[1-^{13}C]methionine and L-[C^2H_3]methionine. A lower rate of methionine transmethylation in NASH compared with control subjects was observed, suggesting that hepatic MAT activity is impaired in these subjects (105). Hypermethioninemia also occurs in *Mat1a* KO mice, confirming the importance of MAT1A in methionine clearance (144). *Mat1a* KO mice appear normal at a young age but are more susceptible to steatosis induced by a choline-deficient diet and liver injury induced by CCl_4, and they develop NASH and HCC spontaneously on a normal diet by 8 and 18 mo, respectively (144, 154). The following summarizes the current understanding of pathways that are abnormal in this model that may contribute to the phenotype observed (FIGURE 7).

A. Increased Oxidative Stress

Mat1a KO mice have increased hepatic oxidative stress (154). There are several contributing factors. The first is decreased hepatic GSH level as AdoMet is an important precursor for GSH through the trans-sulfuration pathway (142). *Mat1a* KO mice have 75 and 40% lower hepatic AdoMet and GSH levels, respectively (144). In addition, there is impaired mitochondrial function and enhanced CYP2E1 activity (154, 227). Thus *Mat1a* KO mice are more susceptible to liver injury induced by CCl_4 (154), a hepatotoxin that requires CYP2E1 to mediate its toxicity (262). Interestingly, AdoMet has been shown to be a noncompetitive inhibitor of CYP2E1 catalytic activity, and depletion of AdoMet sensitizes hepatocytes to CYP2E1 toxicity in vitro while exogenous AdoMet protects against CYP2E1-dependent hepatotoxicity in vivo (35). Regarding impaired mitochondrial function, one of the mechanisms is reduced protein level of prohibitin 1 (PHB1), a well-known mitochondrial

chaperone protein (175, 183). PHB1 is one of the proteins found to be downregulated from the time of birth to development of NASH at 8 mo (227). PHB1 is essential for mitochondrial function, and liver-specific *Phb1* KO mice have significant liver injury at a young age with markedly abnormal mitochondria and increased oxidative stress (113). Mice lacking *Phb1* develop progressive fibrosis and multi-focal HCC by ~8–10 mo of age (113).

Reduced PHB1 protein level is also found in obese patients and *ob/ob* mice (227). A possible role of PHB1 in HCC development is discussed further below in section VII*G*.

B. Abnormal Lipid Homeostasis

Mat1a KO mice have abnormal hepatic lipid homeostasis as evident by increased susceptibility to steatosis induced by a choline-deficient diet and spontaneous development of NASH (144, 154). Recently, the role of MAT1A in very-low-density lipoprotein (VLDL) assembly was investigated. Loss of MAT1A increased the number of VLDL (apoB) particles secreted by 3-mo-old KO mice but decreased the mobilization of hepatic triglycerides (TG) into these particles resulting in lipoproteins that contained less TG and were smaller in diameter (30). Plasma lipid homeostasis was also altered with an increase in lipid transport in low-density lipoprotein (LDL) subclasses and decrease in high-density lipoprotein (HDL) subclasses. Importantly, these abnormalities were corrected after AdoMet treatment for 7 days (30), which normalized hepatic AdoMet levels in these mice (241). Of interest, rats fed a choline-deficient diet, which would deplete hepatic AdoMet content, were noted over 40 years ago to have impaired release of hepatic TGs (139). In addition, recently Walker et al. (259) reported acute knockdown of *MAT1A* resulted in activation of SREBP-1a, which would enhance lipogenesis (see also sect. IX*E*). Taken together, normal hepatic AdoMet level is essential to maintain normal lipid homeostasis.

C. Increased Genomic Instability

Several abnormal pathways have been identified in *Mat1a* KO mice that can contribute to HCC formation. One is increased genomic instability (GI) (241), which is the result of chromosomal rearrangements, duplications, genomic mutations, deletion, and replication errors (49). Acquisition of GI is thought to occur in the early process of carcinogenesis (49). Recently Calvisi et al. (28) showed that early changes in methionine/AdoMet metabolism (fall in AdoMet) and global DNA hypomethylation have a prognostic value for hepatocarcinogenesis in most patients possibly acting through a modulation of GI. Multiple cellular pathways defend against GI; one of the most critical is the apurinic/apyrimidinic endonuclease 1 (APEX1), which is a multifunctional protein possessing both DNA repair and redox regulatory activities (241). Oxidative stress induces APEX1 expression, which is an adaptive response to defend against GI (211). Since *Mat1a* KO mice have increased oxidative stress, one would expect higher APEX1 expression. However, APEX1 expression was actually downregulated in *Mat1a* KO mice livers despite increased GI (241). Both mRNA and protein levels of APEX1 were lower in *Mat1a* KO livers, with the protein level more reduced than the mRNA level, suggesting posttranslational regulation. A useful in vitro model to examine the consequences of MAT1A downregulation and AdoMet depletion is cultured primary hepatocytes, as they rapidly dedifferentiate and lose *MAT1A* expression (241). Supplementing culture medium with AdoMet can prevent AdoMet depletion, blunt the fall in *MAT1A* expression, and completely prevent the fall in APEX1 protein level (241). Thus an important consequence of AdoMet depletion is decreased APEX1 protein stability and increased GI, possibly facilitating malignant degeneration.

D. Dysregulated ERK Signaling

AdoMet's ability to stabilize APEX1 is not unique. In subsequent work, Tomasi et al. (243) found that AdoMet also stabilizes dual-specificity phosphatase 1 (DUSP1) (243). DUSP1

belongs to a family of dual-specificity MAPK phosphatases that can dephosphorylate both serine/threonine and tyrosine residues and control MAPK signaling activity (244). In highly malignant HCC, ERK is overexpressed while DUSP1 expression is decreased (243). DUSP1 and ERK regulate each other in a reciprocal manner. Prolonged ERK activation promotes phosphorylation at the Ser296 residue of DUSP1, which renders the DUSP1 protein susceptible to proteasomal degradation (136). In contrast, transient ERK activation activates DUSP1, which in turn shuts off ERK by dephosphorylation (136). *Mat1a* KO mice livers have increased basal ERK activity (42, 243). On the other hand, hepatic DUSP1 expression is low in *Mat1a* KO mice both at the mRNA and protein level, with protein level lower than mRNA (243). AdoMet treatment corrected the AdoMet deficiency in *Mat1a* KO mice and normalized DUSP1 expression (243). AdoMet normalized DUSP1 mRNA level likely by enhancing p53 binding to its consensus element in the *DUSP1* promoter, which is known to activate *DUSP1* transcriptionally (243). AdoMet also stabilized DUSP1 protein by inhibiting the proteasomal activity, which was increased in *Mat1a* KO livers (243). Importantly, with normalization of DUSP1 expression, ERK activity returned to that of control level (243). Thus AdoMet deficiency can lead to uncontrolled ERK activity due at least in part to decreased DUSP1 expression.

It should be noted that there are conflicting data on the effect of AdoMet on proteasomal activity. Osna et al. (188) reported that ethanol's inhibitory effect on proteasomal activity in cultured cells may be related to lower AdoMet/AdoHcy ratio, and AdoMet treatment prevented this. French et al. (71) also reported that in an animal model of diet-induced formation of Mallory-Denk bodies (MDBs), there is a shift from 26S proteasome to immunoproteasome formation, which was prevented by AdoMet administration. In our study, incubation of purified 26S proteasomes with AdoMet led to rapid degradation of some of the 26S proteasomal subunits, which was blocked by the proteasome inhibitor MG132 (243). This suggests either AdoMet activates the intrinsic protease activity of the proteasome, leading to degradation of some of its subunits, or it modifies the proteasome posttranslationally, which makes the protein more susceptible to protease degradation. D'Anselmi et al. (53) have also reported a similar inhibition by AdoMet on proteasomal activity. The explanation for the conflicting report is unclear at present.

E. Dysregulated LKB1/AMPK Signaling

In addition to enhanced ERK signaling, *Mat1a* KO mice livers also exhibit enhanced LKB1 and AMPK activity (250, 253). Although AMPK and LKB1 are traditionally thought of as metabolic tumor suppressors, they induce proliferation in hepatocytes following HGF stimulation, and AdoMet can block this (253) (see sect. IV*A*). AMPK activation in hepatocytes results in nuclear to cytoplasmic HuR translocation, which leads to stabilization of several cyclin mRNAs to enhance growth (FIGURE 5). AMPK can also activate eNOS, leading to NO formation, which can inactivate MAT I/III and result in lowering of AdoMet level and releasing the inhibitory tone exerted on mitogens (253). Recently, the increase in LKB1 activity in HCC cells derived from *Mat1a* KO livers was found to be required for cell survival, and increased LKB1 activity was also found in human HCC samples (160). Whether increased LKB1 contributes to tumor growth survival in other models of hepatocarcinogenesis or is specific of the *Mat1a* KO is not known.

F. Cancer Stem Cell Expansion

In recent years the role of cancer stem cells (or tumor initiating stem cells) has received increasing attention in the pathogenesis of many cancers, including HCC (121, 230). Hepatic cancer stem cells can be derived from hepatic stem/progenitor cells (also known as oval cells) and dedifferentiation of matured hepatocytes, although there is no evidence for the latter (121). Oval cells reside near the terminal bile ducts, at the hepatocyte-cholangiocyte

interface. Oval cells are quiescent and few in number in adult normal liver and proliferate only during severe, prolonged liver injury and in models of experimental carcinogenesis (221). *Mat1a* KO mice have expansion of oval cells as they age (221). A sub-population of these oval cells stains positive for CD133, a cancer stem cell marker, and they are tumorigenic when injected into immune-deficient mice (221). This was the first demonstration of adult liver stem cells possessing tumorigenic potential in a model without using carcinogenic treatment or manipulating the expression of tumor-suppressors or oncogenes. The cancer stem cells from *Mat1a* KO mice possess highly enhanced MAPK signaling with increased ERK activity (59), similar to *Mat1a* KO livers (42, 243). These cancer stem cells use their constitutive ERK activation to evade the apoptotic effect of TGF-β, a well-known growth inhibitor in hepatocytes (59, 184). How AdoMet deficiency allows expansion of oval cells remains unknown.

G. Decreased Prohibitin 1 Expression

Using proteomics, prohibitin 1 (PHB1) was identified as one of several mitochondrial proteins downregulated in *Mat1a* KO livers from birth until development of NASH (227). Similar to APEX1 and DUSP1, AdoMet also stabilizes PHB1 protein during culture of hepatocytes (227). PHB1 belongs to an evolutionary conserved and ubiquitously expressed family of proteins with a variety of suggested activities in different cellular compartments (175, 183). Two homologous PHB proteins, PHB1 and PHB2, form a large multimeric complex that is found largely in the inner mitochondrial membrane where it exerts a chaperone-like function to stabilize newly synthesized mitochondrial proteins (182) and maintains the organization and stability of mitochondrial nucleoids (106). They are essential for mitochondrial function and biogenesis in yeast (235) and mammals (172). PHB1 is also found in the nucleus, where it has been shown to interact with Rb and p53 among other proteins to bring about a change in transcriptional activities of E2F (259) and p53 (74). These nuclear events have been associated with inhibition of cell cycle progression (260) and induction of apoptosis (74). PHB1 was originally cloned from regenerating livers (its expression was found to be nearly absent shortly after 2/3 partial hepatectomy), identified as having antiproliferative activity and thought to be a tumor suppressor (hence its name) (168). However, the tumor suppressor activity of PHB1 is highly controversial as PHB1 expression is actually higher in many transformed cells and tumors (183). Deletion of PHB1 leads to embryonic lethality so that its in vivo function was unexplored until the development of liver-specific *Phb1* KO mice (113). There is significant fetal wastage and biochemical and histological liver injury at 3 wk of age. The KO livers have markedly abnormal mitochondria and exhibit increased oxidative stress, progressive fibrosis, and hepatocyte dysplasia. From 20 wk on, KO mice have multiple liver nodules, and from 35 to 46 wk, 38% have multifocal HCC. Since HCC may have resulted from chronic injury and inflammation, the effect of acute PHB1 knockdown was also examined in murine nontransformed AML12 cells. Knockdown by 30% at the protein level raised cyclin D1, H19, and IGF2 expression as well as increased E2F binding to the cyclin D1 promoter and proliferation. The opposite occurred with PHB1 overexpression (113). These results support PHB1 as a tumor suppressor at least in hepatocytes.

VIII. CONSEQUENCES OF CHRONIC HEPATIC AdoMet EXCESS

GNMT expression is reduced or absent in HCC (43) and downregulated in the livers of patients at risk of developing HCC, such as in those with hepatitis C virus- and alcohol-induced cirrhosis (7). Moreover, a loss of heterozygosity of *GNMT* has been reported in ~40% of HCC patients, and *GNMT* has been proposed to be a tumor-susceptibility gene for liver cancer (43, 246). Identification of several children with mutations of GNMT as having mild to moderate liver disease with elevated serum aminotransferases (6, 178) further suggests that a defect in this enzyme leads to liver disease. Two *Gnmt* KO mouse models

have been developed (133, 148, 158). These mouse models have provided much insight into the consequences of chronic hepatic AdoMet excess and altered signaling pathways that may lead to malignant degeneration. Both mouse models appear normal at birth but develop HCC spontaneously, although at different ages (133, 158). The *Gnmt* KO mouse model generated by Luka et al. (158) develops NASH and HCC at 3 and 8 mo of age, respectively, whereas the *Gnmt* KO model generated by Liao et al. (133) develops HCC at 17 mo of age. The reason for these differences is not clear. The following summarizes the current understanding of pathways that are abnormal in *Gnmt* KO mice that may contribute to the phenotype observed (FIGURE 8).

A. Impaired Lipid Metabolism

In *Gnmt* KO mice, the expression of peroxisome proliferator-activated receptor alpha (*PPARα*), a lipid-activated transcription factor primarily expressed in the liver that activates fatty acid β-oxidation (126), is downregulated, and the activity of AMPK, a key regulator of cellular energy that once activated switches on the oxidation of fatty acids while switching off the synthesis of lipids (84), is inhibited (252). Additionally, the expression of *CD36*, a fatty acid translocase whose elevated expression is associated with increased hepatic fatty acid uptake (115); *ADFP*, a gene related to hepatic lipid droplet formation and whose absence relieves steatosis (37); *PPARγ*, a transcription factor whose overexpression in the liver induces steatosis (249); and of the *Cyp4a* omega-oxidation system that metabolizes long-chain and very long-chain fatty acids generating H_2O_2 and toxic dicarboxylic acids in the process (216) are up-regulated in *Gnmt* KO mice (252). Serum bile acids, which may contribute to the higher hepatic fatty acid oxidation in NASH (55), are also elevated in *Gnmt* KO mice (13). Thus both a deficiency and an excess of AdoMet can lead to uncontrolled hepatic lipid metabolism, steatosis, and oxidative stress, albeit with distinct mechanisms (see sect. VII*B*). How AdoMet excess influences the expression of these genes involved in lipid metabolism is unclear.

B. DNA and Histone Hypermethylation

As in human HCC, the Ras and JAK/STAT pathways are activated in *Gnmt* KO mice liver, and also as found in human HCC, activation of these signaling pathways coincided with the downregulation of the expression of the Ras and JAK/STAT inhibitors *Rassf1, Rassf4*, *Socs1*, *Socs2*, *Socs3*, and *Cis* (158). These inhibitors of the Ras and JAK/ STAT signaling pathways, which are frequently inactivated by promoter methylation, have been involved in human carcinogenesis (27). Hypermethylation of *Rassf1* and *Socs2* promoters, and trimethylation of histone 3 lysine 27 (H3K27) bound to *Rassf1* and *Socs2* promoters, has been identified in livers from *Gnmt* KO mice. Moreover, and consistent with its function as a cellular AdoMet buffer that prevents the occurrence of aberrant methylation reactions, *Gnmt* KO mice show global liver DNA hypermethylation (158). Thus absence of GNMT results in increased hepatic AdoMet and aberrant DNA and histone hypermethylation, which can lead to major epigenetic alteration in gene expression regulation.

Consistent with these findings, treatment of *Gnmt* KO mice with nicotinamide, a substrate of nicotinamide *N*-methyltransferase (NNMT, an enzyme mainly expressed in the liver), leads to the reduction of hepatic AdoMet content, normalization of lipid metabolism, and prevention of global DNA methylation and of the activation of the Ras and JAK/STAT pathways, fatty liver, and fibrosis formation (252).

C. Expansion of Hepatic Progenitor Cells

Liver regeneration is inhibited in *Gnmt* KO mice as a result of a sustained increase in AdoMet content (251). The concept of intrahepatic stem/progenitor cells activation to replicate and differentiate to replenish the liver parenchyma when regeneration is suppressed

is well established (153). With time, these intrahepatic stem/progenitor cells, which are resistant to liver injury, may selectively proliferate and form preneoplastic nodules that ultimately form liver tumors. Consistent with this concept, stem/progenitor cells staining positive for antibodies to oval cells were visualized in livers of *Gnmt* KO mice at 8 mo of age (157). Thus both a deficiency and an excess of AdoMet can lead to expansion of liver stem/progenitor cells (see sect. VII*F*). Whether there are also cancer stem cells among the progenitor cells in the *Gnmt* KO livers remains to be examined. How abnormal hepatic AdoMet content sets the stage for normally dormant stem/progenitor cells to replicate and differentiate is not known.

IX. THERAPEUTIC USE OF AdoMet IN LIVER INJURY

AdoMet treatment is well established to ameliorate liver injury in multiple animal models, including galactosamine, acetaminophen, alcohol, thioacetamide, endotoxemia, CCl_4, bile duct ligation (BDL), NASH, and ischemia-reper-fusion (34, 35, 114, 162, 165, 273, 274). AdoMet not only protected against acute injury, it also reduced fibrosis in multiple experimental models (35, 165, 274). The underlying mechanisms include AdoMet's ability to raise GSH level (particularly in the mitochondria), inactivate CYP2E1, suppress TNF-α expression, maintain mitochondrial function at least in part via membrane dynamics and changes in mitochondrial proteome, protect hepatocytes against apoptosis, and suppress collagen synthesis by HSCs (3, 22, 35, 164) (see sect. IV*B*). In hepatocytes, AdoMet not only serves as a precursor for GSH synthesis, it is also able to positively regulate the expression of glutamate-cysteine ligase catalytic (GCL), the rate-limiting enzyme in GSH synthesis (114, 274). Thus, through its multiple actions, AdoMet has been a well-known hepatoprotective agent in experimental liver injury.

Evidence for AdoMet's efficacy in human liver disease is much more limited. In humans, AdoMet is available in oral and parenteral formulations. Bioavailability is much better with parenteral administration. AdoMet was found to be 80–90% bioavailable after an intramuscular injection, with a half-life of 81 min after 100 mg injected dose and 101 min after a 500 mg dose (85). In the United States, AdoMet is only available as an oral dietary supplement, while it is a prescribed medication in Russia, India, China, Italy, Germany, Vietnam, and Mexico (information provided by María Roche, International Pharmaceuticals Abbott). Bio-availabilty of the orally administered AdoMet is poor due to a significant first-pass effect and rapid hepatic metabolism. Peak plasma concentrations of 0.5–1 mg/l are achieved 3–5 h after single AdoMet doses ranging from 400 to 1,000 mg and decline to baseline within 24 h (85). One study showed a gender difference in bioavailability, with women showing three- to sixfold greater peak plasma values than men (85). Plasma-protein binding of AdoMet is no more than 5%, and it crosses the blood-brain barrier, with slow accumulation in the cerebrospinal fluid. Unmetabolized AdoMet is excreted in urine and feces. Thus far, no significant adverse events have been reported in clinical trials using either parenteral or enteral AdoMet (85). However, there is a theoretical concern of administering AdoMet to human immune deficiency (HIV)-infected and other immunocompromised individuals at risk for *Pneumocystis carinii* infection. This is because *P. carinii* is an AdoMet auxotroph so that AdoMet administration enhanced its growth and exacerbated the infection in animal models (171). *P. carinii* actively transports AdoMet, and targeting AdoMet transporters is being proposed as a potential therapy (202). Some species of *Rickettsia* and *Leishmania* also do not synthesize AdoMet (202), and it is possible that AdoMet administration could exacerbate these infections as well. The only eukaryotic plasma membrane AdoMet transporters characterized to date are the *Saccharomyces cerevisiae* SAM3 protein, belonging to the amino acid permease superfamily (220), and the *Leishmania* AdoMet transporter (AdoMetT1) that belongs to the folate biopterin transporter family (60). In mammalian cells, transport of AdoMet across the plasma membrane to the

blood occurs when intracellular AdoMet abnormally accumulates as in *Gnmt* KO mice and in patients with GNMT or AHCY deficiency (6, 11, 12, 21, 82, 158, 178, 258). This transport mechanism probably operates in the opposite direction after either parenteral or enteral AdoMet administration, or during cell incubation with high AdoMet. The proteins mediating AdoMet transport across the plasma membrane in mammalian cells are not known. Mitochondrial transporters of AdoMet (SAMC) in yeast and mammalian cells have been also functionally characterized. They belong to the mitochondrial carrier protein family (MCF), a class of proteins mediating the transport of various substrates (1, 152). As AdoMet is not synthesized in the mitochondria, the main function of SAMC is probably to facilitate the uptake of AdoMet into the mitochondria in exchange for internal AdoHcy generated by MTs, which is hydrolyzed exclusively in the cytoplasm.

Below are several nonmalignant human liver diseases where AdoMet treatment may be of benefit.

A. Cholestasis of Pregnancy

The Agency for Healthcare Research and Quality (AHRQ) reviewed 102 individual clinical trials of AdoMet in 2002 (85). Forty-one studies were in liver diseases and six were included in a meta-analysis of the efficacy of AdoMet to relieve pruritus and decrease elevated serum bilirubin levels associated with cholestasis of pregnancy (85). AdoMet treatment (mostly 800 mg iv) was associated with a significant decrease in pruritus and serum bilirubin levels compared with placebo (85). AdoMet did not appear to be superior to ursodeoxycholic acid (UDCA), the conventional therapy, and combining AdoMet with UDCA in some studies appeared to be better than either alone (85).

B. Alcoholic Liver Disease

Abnormal methionine metabolism and reduced hepatic AdoMet levels are well established in animal models as well as patients with alcoholic liver disease (ALD) (123, 247). In 2001, the Cochrane Hepato-Biliary Group analyzed 8 clinical trials with a total of 330 ALD patients treated with AdoMet (215). However, most of the studies were small with variable quality, and the meta-analysis did not find a significant benefit of AdoMet treatment. Only 1 trial with 123 patients with alcoholic cirrhosis used adequate methodology and reported clearly on mortality and liver transplantation (163). In this study patients were randomized to receive AdoMet or placebo at 1,200 mg/day in three divided doses for 2 years. Mortality decreased from 30% in the placebo group to 16% in the AdoMet group ($P = 0.077$), and if patients with more advanced cirrhosis (Child's class C) were excluded (8 patients), the mortality was significantly less in the AdoMet group (12%) compared with the placebo group (25%, $P = 0.025$). However, this was a subgroup analysis that was data driven so that confirmation with another larger placebo-controlled trial is needed. A smaller trial using the same dosage of AdoMet in less advanced ALD patients showed no benefit of AdoMet on biochemical parameters or histology after 24 wk (169). However, the study included only 24 subjects, and a post-liver biopsy was done in 14 of them due to the high drop-out rate. This along with the short duration of the trial precluded any conclusion to be made.

C. Chronic Hepatitis C Treatment

Recent data suggest AdoMet improves interferon signaling and early viral response to HCV treatment (63, 66). Potential mechanism(s) include increasing STAT1 methylation to enhance STAT1-DNA binding and greater IFN-stimulated gene responses (63). Interestingly, the expression of viperin, one of the IFN-stimulated genes, predicts response to pegylated-IFN-α/ribavirin treatment (228). Since AdoMet is also being used to treat depression (85) and depression is a well-known side effect of IFN, AdoMet may be particularly attractive as adjunct to IFN-based therapy in HCV.

D. Potential Use in Chronic Cholestatic Disorders

While the evidence supporting the use of AdoMet in cholestasis of pregnancy is strong (85), there are no randomized controlled trials that examined the efficacy of AdoMet in chronic cholestatic liver diseases. In the United States, cholestatic liver injury is a major cause of chronic liver disease. Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease in which the only Federal Drug Administration-approved medication is UDCA (120). However, UDCA has no proven efficacy against other chronic cholestatic liver diseases such as primary sclerosing cholangitis and even in PBC, only 25–30% of the patients have a complete response (232). Moreover, high-dose UDCA was actually found to be associated with higher risk of developing colon cancer in patients with primary sclerosing cholangitis and ulcerative colitis (62). Therefore, more effective treatment against chronic cholestatic liver diseases is sorely needed.

We recently reported that in mice subjected to BDL, adding AdoMet to UDCA was better than either treatment alone in protecting the liver against BDL-induced chronic liver injury (274). The molecular mechanism may be related to the ability of AdoMet and UDCA to enhance the nuclear binding activity of Nrf2, a key transcription factor that activates many genes involved in defense against oxidative stress via binding to the antioxidant response element (ARE) present in the promoter region of these genes (142, 273, 274). AdoMet and UDCA seem to act by different mechanisms in this regard as they exerted additive effects when combined (273). Some of these ARE-dependent genes are involved in the biosynthesis of GSH, a key antioxidant that is also known to induce choleresis (65). Combined AdoMet and UDCA treatment protected against the fall in GSH biosynthesis during chronic cholestasis (273, 274). Thus an interesting area to examine is whether AdoMet has a role in the treatment of PBC, where UDCA is ineffective in 40% of patients (232), either alone or in combination with UDCA. If AdoMet is effective, then it can be studied in other chronic cholestatic liver diseases as well.

E. Potential Use in NASH

A diet deficient in methyl groups (methionine and choline, also known as the MCD diet) is known to cause hepatic steatosis for almost 100 years (166), and the steatotic effect of ethionine, an inhibitor of AdoMet biosynthesis, is also known for many years (102). Phenotype of the *Mat1a* KO mouse links a reduction in the transmethylation capacity of the liver with hepatic steatosis and liver injury (144, 154). Interestingly, downregulation of *Mat-1* (also known as *sams-1*) in *Caenorhabditis elegans* leads to the accumulation of TG in lipid droplets in the intestine, which was rescued by choline feeding (132), suggesting that the link between AdoMet and fat metabolism is widely extended in nature. Recent elegant work from Walker et al. (259) has provided further insight into the link between AdoMet, transmethylation capacity, and lipogenesis. Their work using *C. elegans*, mammalian cells, and rodent liver suggests reducing transmethylation capacity by various mechanisms (including knocking down *sams-1*) can result in decreased phosphatidylcholine biosynthesis, and this in turn affects Golgi membrane dynamics leading to activation of SREBP-1 and lipogenesis (259). Consistent with these findings, Caballero et al. (22) examined the specific contribution of methionine and choline in the development of NASH using the MCD diet in mice. They found that choline deficiency is responsible for steatosis (TG and free fatty acids accumulation) while methionine deficiency is responsible for weight loss, liver injury, oxidative stress, inflammation, and fibrosis (22). Both methionine-deficient diet and MCD diet resulted in significant depletion of mitochondrial AdoMet and GSH pools, and correcting this depletion with GSH prodrugs as well as AdoMet has been reported to be of benefit (22, 34). Taken together, these findings strongly support the use of AdoMet in NASH, and McClain and colleagues (34) have initiated a National Institutes of Health-

funded clinical trial examining the efficacy of AdoMet in NASH patients, and results are pending.

X. POTENTIAL USE OF AdoMet IN LIVER CANCER

A. Chemoprevention

The idea of using AdoMet in chemoprevention of HCC is not new as several studies from Feo and co-workers from the mid 1980s on demonstrated in several rodent models of hepatocarcinogenesis that AdoMet is effective in preventing HCC (64, 196–198). In these models, hepatic AdoMet level fell, and several protooncogenes became hypomethylated and their expression induced. The mechanism of chemoprevention was thought to be related to preventing the hypomethylation that occurred. Recently AdoMet's chemopreventive effect was examined in a different HCC model where liver cancer cells were directly injected into the liver parenchyma without changing the hepatic AdoMet level. In this model, AdoMet was able to inhibit the establishment of HCC (147). In addition to AdoMet's selective pro-apoptotic effect in liver cancer cells, AdoMet also exhibited anti-angiogenic properties (147). There are more than 500 million people in the world at risk for HCC development (143). Most of these individuals have chronic hepatitis B or C viral infection. The incidence of HCC is expected to rise in the west. Chemoprevention is an important area that has received very little to no attention (143). Given AdoMet's excellent safety profile, abundance of basic science data showing hepatic AdoMet deficiency predisposes to HCC, and well-established efficacy of AdoMet in preventing HCC, it is important to examine AdoMet in both primary and secondary chemoprevention of HCC in humans. Currently there is one phase II clinical trial that is evaluating AdoMet as a potential chemopreventive agent in patients with hepatitis C cirrhosis (177).

B. Treatment

In an orthotopic xenograft HCC model, AdoMet was ineffective in treating already existing HCC as the normal liver was able to upregulate compensatory mechanisms to dispose excess AdoMet (147). Thus chronic delivery of AdoMet at large doses induced the expression of GNMT in the normal liver so that AdoMet could not accumulate (147). This is important as a certain level of AdoMet may be required to exert its pro-apoptotic and anti-angiogenic effect. The caveat is that in human HCC, GNMT is frequently silenced, so it remains to be examined what the outcome of AdoMet treatment is when GNMT is not expressed. While exogenous AdoMet cannot maintain a high hepatic AdoMet level, forced expression of *MAT1A* in a liver cancer cell can (129). This strategy inhibited HCC growth in vitro and in vivo, by inhibiting angiogenesis and promoting apoptosis (129). Two key growth pathways that are upregulated in HCC, ERK and AKT, were inhibited when intracellular AdoMet level was doubled by forcing the expression of *MAT1A* (129). Thus understanding why *MAT1A* is silenced in HCC is important, as increasing *MAT1A* expression in HCC can inhibit HCC growth.

XI. ROLE OF AdoMet OUTSIDE OF THE LIVER

A. Lymphocyte Activation

AdoMet level increases during T-lymphocyte activation due to increased *MAT2A* gene expression and rapid downregulation of the β subunit (83, 240). This was thought to be necessary to provide a source of polyamine biosynthesis, and if AdoMet accumulation is prevented, lymphocyte activation was inhibited (57). More recently, Barve and colleagues (95) found that ethanol inhibited *MAT2A* transcription to result in lower MAT II activity and AdoMet level in T lymphocytes. This sensitized the $CD4^+$ T lymphocytes to caspase-3-dependent activation-induced cell death and may be one of the explanations for alcohol-

induced immune suppression (95). The same group of investigators also found that proliferating and transformed T leukemic cells express higher MAT II activity, and inhibiting MAT II expression in these cells resulted in higher caspase-8-dependent apoptosis (101). These studies support targeting changes in MAT II/AdoMet level as a treatment strategy to either increase, as in the case of ethanol, or inhibit, as in the case of T-cell leukemia. These in vitro studies await confirmation using relevant in vivo models.

B. Depression

The AHRQ also analyzed the efficacy of AdoMet in the treatment of depression (85). They included 28 of 39 studies involving AdoMet treatment in depression in a meta-analysis. When compared with placebo, AdoMet treatment was associated with an improvement of ~6 points in the score of the Hamilton Rating Scale for Depression measured at 3 wk (95% CI 2.2 to 9.0), which was significant statistically and clinically (85). However, AdoMet treatment was not significantly different when compared with conventional antidepressants. The AHRQ concluded, “good dose-escalation studies have not been performed using the oral formulation of AdoMet for depression” and that “additional smaller clinical trials of an exploratory nature should be conducted to investigate uses of AdoMet to decrease the latency of effectiveness of conventional antidepressants and to treat postpartum depression” (85). In light of these studies, the efficacy of AdoMet as an adjunctive treatment for patients who are antidepressant nonresponders has been recently evaluated. In a preliminary open clinical study, AdoMet treatment (400–800 mg/twice daily) was associated with an improvement among 30 outpatients with serotonin reuptake inhibitor (SRI)-resistant major depressive disorder (MDR) (2). More recently, this group carried out a single-center, double-blind, placebo-controlled trial where 73 SRI nonresponders with MDR were treated for 6 wk with AdoMet (800 mg/twice daily) (195). The results of this study suggest that AdoMet can be an effective adjunctive treatment strategy for SRI nonresponders with MDR (180, 195).

C. Arthritis

AHRQ included 10 of 13 studies in osteoarthritis in a meta-analysis of the efficacy of AdoMet to decrease pain of osteoarthritis (85). One large randomized trial showed a decrease in the pain of osteoarthritis with AdoMet treatment compared with placebo and fewer adverse effects compared with treatment with nonsteroidal anti-inflammatory medications without a significant difference in pain reduction. In 2009, the Cochrane Osteoarthritis Group analyzed four clinical trials including 656 patients that compared AdoMet with placebo (224). The Cochrane Group concluded, “the effects of AdoMet on both pain and function may be potentially clinically relevant and, although effects are expected to be small, deserve further clinical evaluation in adequately sized randomized, parallel-group trials in patients with knee or hip osteoarthritis. Meanwhile, routine use of AdoMet should not be advised” (224). Thus, although improving mental and joint health is widely publicized in support of AdoMet use, the overall consensus remains that additional studies are needed.

D. Colon Cancer

Other than HCC, *MAT2A* expression is also induced in human colon cancers and polyps from *Min* mice (41). *MAT2A* induction was required for mitogens like leptin, EGF, and IGF-I to exert their proliferative effect (41). AdoMet and MTA treatment lowered *MAT2A* expression and inhibited the proliferative effect of these mitogens (41). In addition, AdoMet and MTA exerted a selective proapoptotic effect in colon cancer cells in part through down-regulation of cFLIP (both isoforms) and sensitized colon cancer cells to TNF-α-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (131). Recently, TNF-α was shown to play a key role in the development of inflammation-induced colon cancer model in mice

as the TNF-α blocker entanercept reduced the number of colon tumors in this model (205). Since both AdoMet and MTA can inhibit LPS-induced TNF-α expression in macrophages and whole liver (5, 39, 88), we examined whether these agents might be effective in the same model using axozymethane and dextran sulfate sodium to induce colon cancer (130). We found that AdoMet and MTA treatment reduced tumor load by ~40%, which is comparable to several known chemopreventive agents (130). Both treatments inhibited IL-6 expression and IL-6 downstream signaling (STAT3 activation and IL-10) and NFκB and AKT activation, all key pathways that participate in colon carcinogenesis. MTA treatment also reduced the expression of several pro-survival genes such as cFLIP, MAT2A, MAT2B, cyclooxygenase-2, and IL-1β. Interestingly, neither agent exerted any influence on TNF-α or iNOS mRNA levels, even though both were shown to inhibit their expression induced by LPS in macrophages or liver (5, 39). In addition, AdoMet pre-treatment raised hepatic IL-10 level following LPS (114), but in the colon cancer model, it lowered IL-10 level. Thus the effect of these agents on various cytokines and proinflammatory mediators is very much tissue specific. In vivo, both treatments induced apoptosis but inhibited proliferation and β-catenin activation on immunohistochemistry (130). Taken together, AdoMet and MTA appear to target multiple signaling pathways known to be important in colon carcinogenesis. They may have a potential role as chemopreventive agents in patients at risk for colon cancer (such as those with inflammatory bowel disease). AdoMet has a high safety profile, making it particularly attractive. MTA is not yet available for human use but is being developed into a drug by a pharmaceutical company in Spain.

XII. SUMMARY AND FUTURE DIRECTIONS

AdoMet, derived from methionine catabolism in all mammalian cells, is not only the principal methyl donor, but also a precursor of *1*) GSH, the major endogenous antioxidant; *2*) polyamines, a critical group of molecules involved in the regulation of transcription, translation, cell growth, and apoptosis; *3*) MTA, a byproduct of spontaneous AdoMet degradation and polyamine synthesis that mimics many of AdoMet's actions when given in pharmaceutical doses; and *4*) 5′-deoxyadenosyl 5′-radical, which can initiate a radical reaction by removing a hydrogen atom from a variety of substrates including proteins and nucleic acids. From NASH to cirrhosis and HCC, methionine metabolism is increasingly impaired. Accordingly, the expression of *MAT1A* and *GNMT*, the main two genes involved in hepatic AdoMet synthesis and catabolism, respectively, are markedly diminished in patients with liver cirrhosis and minimal to absent in HCC. *Mat1a*- and *Gnmt*-KO mice spontaneously develop NASH and HCC, indicating that the loss in the expression of these two genes in liver cancer is the cause of the carcinogenic process and not its consequence. Downregulation of *MAT1A* and *GNMT* in HCC is accompanied by an increased expression of *MAT2A* and *MAT2B*. The accumulating evidence indicates that this switch in the expression of *MAT* and *GNMT* genes affects multiple pathways, some of which may be independent of AdoMet. Future research should focus on identifying molecular mechanisms responsible for how dysregulation of *MAT*/*GNMT* gene expression leads to oxidative stress, impaired lipid metabolism, and malignant transformation. Finally, AdoMet treatment has been shown to ameliorate liver injury in multiple animal models. Although there is evidence for its efficacy in humans, new clinical trials are necessary to unambiguously establish its benefit in several nonmalignant liver diseases such as NASH, chronic cholestatic disorders, alcoholic liver cirrhosis, and hepatitis C, as well as in the chemoprevention of HCC. The therapeutic potential of MTA in both HCC and colon cancer should be also investigated.

Acknowledgments

GRANTS This work was supported by National Institutes of Health Grants R01DK51719 (to S. C. Lu), R01DK092407 (to S. C. Lu), P30DK48522 (to S. C. Lu), R01AT1576 (to S. C. Lu and J. M. Mato), and

R01AT004896 (to S. C. Lu and J. M. Mato) as well as Plan Nacional of I+D SAF 2011–29851 and Departamento de Educación Gobierno Vasco 2011 (to J. M. Mato).

REFERENCES

1. Agrimi G, Di Noia MA, Marobbio CM, Fiermonte G, Lasorsa FM, Palmieri F. Identification of the human mitochondrial *S*-adenosylmethionine transporter: bacterial expression, reconstitution, functional characterization and tissue distribution. Biochem J. 2004; 379:183–190. [PubMed: 14674884]
2. Alpert JE, Papakostas G, Mischoulon D, Worthington JJ 3rd, Petersen T, Mahal Y, Burns A, Bottiglieri T, Nierenberg AA, Fava M. *S*-adenosyl-l-methionine (SAMe) as an adjunct for resistant major depressive disorder: an open trial following partial or nonresponse to selective serotonin reuptake inhibitors or venlafaxine. J Clin Psychopharmacol. 2004; 24:661–664. [PubMed: 15538131]
3. Andringa KK, King AL, Eccleston HB, Mantena SK, Lander A, Jhala NC, Dickinson DA, Squadrito GL, Bailey SM. Analysis of the liver mitochondrial proteome in response to ethanol and *S*-adenosylmethionine treatments: novel molecular targets of disease and hepatoprotection. Am J Physiol Gastrointest Liver Physiol. 2010; 298:G732–G745. [PubMed: 20150243]
4. Ansorena E, García-Trevijano ER, Martínez-Chantar ML, Huang ZZ, Chen L, Mato JM, Iraburu M, Lu SC, Avila MA. *S*-adenosylmethionine and methylthioadenosine are antiapoptotic in cultured rat hepatocytes but proapoptotic in human hepatoma cells. Hepatology. 2002; 35:274–280. [PubMed: 11826399]
5. Ara AI, Xia M, Ramani K, Mato JM, Lu SC. *S*-adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation. Hepatology. 2008; 47:1655–1666. [PubMed: 18393372]
6. Augoustides-Savvopoulou P, Luka Z, Karyda S, Stabler SP, Allen RH, Patsiaoura K, Wagner C, Mudd SH. Glycine *N*-methyltransferase deficiency: a new patient with a novel mutation. J Inherit Metab Dis. 2003; 26:745–759. [PubMed: 14739680]
7. Avila MA, Berasain C, Torres L, Martin-Duce A, Corrales FJ, Yang HP, Prieto J, Lu SC, Caballeria J, Rodes J, Mato JM. Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma. J Hepatol. 2000; 33:907–914. [PubMed: 11131452]
8. Avila MA, Carretero MV, Rodriguez EN, Mato JM. Regulation by hypoxia of methionine adenosyltransferase activity and gene expression in rat hepatocytes. Gastroenterology. 1998; 114:364–371. [PubMed: 9453498]
9. Avila MA, García-Trevijano ER, Lu SC, Corrales FJ, Mato JM. Methylthioadenosine. Int J Biochem Cell Biol. 2004; 36:2125–2130. [PubMed: 15313459]
10. Avila MA, Mingorance J, Martínez-Chantar ML, Casado M, Martín-Sanz P, Bascá L, Mato JM. Regulation of rat liver *S*-adenosylmethionine synthetase during septic shock: role of nitric oxide. Hepatology. 1997; 25:391–396. [PubMed: 9021952]
11. Barić I, Cuk M, Fumić K, Vugrek O, Allen RH, Glenn B, Maradin M, Pazanin L, Pogribny I, Rados M, Sarnavka V, Schulze A, Stabler S, Wagner C, Zeisel SH, Mudd SH. *S*-Adenosylhomocysteine hydrolase deficiency: a second patient, the younger brother of the index patient, outcomes during therapy. J Inherit Metab Dis. 2005; 28:885–902. [PubMed: 16435181]
12. Barić I, Fumic K, Glenn B, Cuk M, Schulze A, Finkelstein JD, James SJ, Mejaski-Bosnjak V, Pazanin L, Pogribny IP, Rados M, Sarnavka V, Scukanec-Spoljar M, Allen RH, Stabler S, Uzelac L, Vugrek O, Wagner C, Zeisel S, Mudd SH. *S*-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. Proc Natl Acad Sci USA. 2004; 101:4234–4239. [PubMed: 15024124]
13. Barr J, Vázquez-Chantada M, Alonso C, Pérez-Cormenzana M, Mayo R, Galán A, Caballería J, Martín-Duce A, Tran A, Wagner C, Luka Z, Lu SC, Castro A, Le Marchand-Brustel Y, Martínez-Chantar ML, Veyrie N, Clément K, Tordjman J, Gual P, Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. J Proteome Res. 2010; 9:4501–4512. [PubMed: 20684516]

14. Basu I, Cordovano G, Das I, Belbin TJ, Guha C, Schramm VL. A transition state analogue of 5′-methylthioadenosine phosphorylase induces apoptosis in head and neck cancers. J Biol Chem. 2007; 282:21477–21486. [PubMed: 17548352]
15. Basu I, Locker J, Cassera MB, Belbin TJ, Merino EF, Dong X, Hemeon I, Evans GB, Guha C, Schramm VL. Growth and metastases of human lung cancer are inhibited in mouse xenografts by a transition state analogue of 5′-methylthioadenosine phosphorylase. J Biol Chem. 2011; 286:4902–4911. [PubMed: 21135097]
16. Beaudouin C, Haurat G, Laffitte JA, Renaud B. The presence of (+)-*S*-adenosyl-l-methionine in the rat brain and its lack of effect on phenylethanolamine *N*-methyltransferase activity. J Neurochem. 1993; 61:928–935. [PubMed: 8360691]
17. Bentley R. Role of sulfur chirality in the chemical processes of biology. Chem Soc Rev. 2005; 34:609–624. [PubMed: 15965542]
18. Bertino JR, Waudd WR, Parker WB, Lubin M. Targeting tumors that lack methylthioadenosine phosphorylase (MTAP) activity. Cancer Biol Ther. 2011; 11:627–631. [PubMed: 21301207]
19. Boise LH, González-García M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nuñez G, Thompson CB. Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell. 1993; 74:597–608. [PubMed: 8358789]
20. Borowiak M, Garratt AN, Wüstefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. Proc Natl Acad Sci USA. 2004; 101:10608–10613. [PubMed: 15249655]
21. Buist NR, Glenn B, Vugrek O, Wagner C, Stabler S, Allen RH, Pogribny I, Schulze A, Zeisel SH, Barić I, Mudd SH. *S*-adenosylhomocysteine hydrolase deficiency in a 26-year-old man. J Inherit Metab Dis. 2006; 29:538–545. [PubMed: 16736098]
22. Caballero F, Fernández A, Matías N, Martínez L, Fucho R, Elena M, Caballeria J, Morales A, Fernández-Checa JC, García-Ruiz C. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis. Impact on mitochondrial *S*-adenosyl-l-methionine and glutathione. J Biol Chem. 2010; 285:18528–18536. [PubMed: 20395294]
23. Cabrero C, Duce AM, Ortiz P, Alemany A, Mato JM. Specific loss of the high-molecular weight form of *S*-adenosyl-l-methionine synthetase in human liver cirrhosis. Hepatology. 1988; 8:1530–1534. [PubMed: 3192166]
24. Cabrero C, Puerta J, Alemany S. Purification and comparison of two forms of *S*-adenosyl-l-methionine synthetase from rat liver. Eur J Biochem. 1987; 170:299–304. [PubMed: 3121322]
25. Cai J, Mao Z, Hwang JJ, Lu SC. Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. Cancer Res. 1998; 58:1444–1450. [PubMed: 9537246]
26. Cai J, Sun W, Hwang JJ, Stain S, Lu SC. Changes in *S*-adenosylmethionine synthetase in human liver cancer: molecular characterization and significance. Hepatology. 1996; 24:1090–1097. [PubMed: 8903381]
27. Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology. 2006; 130:1117–1128. [PubMed: 16618406]
28. Calvisi DF, Simile MM, Ladu S, Pellegrino R, De Murtas V, Pinna F, Tomasi ML, Frau M, Virdis P, De Miglio MR, Muroni MR, Pascale RM, Feo F. Altered methionine metabolism and global DNA methylation in liver cancer: relationship with genomic instability and prognosis. Int J Cancer. 2007; 121:2410–2420. [PubMed: 17680562]
29. Cannon LM, Butler FN, Wan W, Zhou ZS. A stereospecific colororimetric assay for (*S,S*)-adenosyl methionine quantification bases on thiopurine methyltransferase-catalyzed thiol methylation. Anal Biochem. 2002; 308:358–363. [PubMed: 12419350]
30. Cano A, Buqué X, Martínez-Uña M, Aurrekoetxea I, Menor A, García-Rodriguez JL, Lu SC, Martínez-Chantar ML, Mato JM, Ochoa B, Aspichueta P. Methionine adenosyltransferase 1A gene deletion disrupts hepatic VLDL assembly in mice. Hepatology. 2011; 54:1975–1986. [PubMed: 21837751]
31. Cantoni GL. The nature of the active methyl donor formed enzymatically from l-methionine and adenosinetriphosphate. J Am Chem Soc. 1952; 74:2942–2943.

32. Cantoni GL. Biochemical methylations: selected aspects. Annu Rev Biochem. 1975; 45:285–306.
33. Castro A, Le Marchand-Brustel Y, Martínez-Chantar ML, Veyrie N, Clément K, Tordjman J, Gual P, Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. J Proteome Res. 2010; 9:4501–4512. [PubMed: 20684516]
34. Cave M, Deaciuc I, Mendez C, Song Z, Joshi-Barve S, Barve S, McClain C. Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. J Nutr Biochem. 2007; 18:184–195. [PubMed: 17296492]
35. Cederbaum AI. Hepatoprotective effects of *S*-adenosyl-l-methionine against alcohol- and cytochrome P450 2E1-induced liver injury. World J Gastroenterol. 2010; 16:1366–1376. [PubMed: 20238404]
36. Chalfant CE, Rathman K, Pinkerman RL, Wood RE, Obeid LM, Ogretmen B, Hannun YA. De novo ceramide regulates the alternative splicing of caspase 9 and Bcl-x in A549 lung adenocarcinoma cells. J Biol Chem. 2002; 277:12587–12595. [PubMed: 11801602]
37. Chang BH, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, Chan L. Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. Mol Cell Biol. 2006; 26:1063–1076. [PubMed: 16428458]
38. Chawla RK, Jones DP. Abnormal metabolism of *S*-adenosyl-l-methionine in hypoxia rat liver. Similarities to its abnormal metabolism in alcoholic cirrhosis. Biochim Biophys Acta. 1994; 1199:45–51. [PubMed: 8280752]
39. Chawla RK, Watson WH, Eastin CE, Lee EY, Schmidt J, McClain CJ. *S*-adenosylmethionine deficiency and TNFα in lipopolysaccharide-induced hepatic injury. Am J Physiol Gastrointest Liver Physiol. 1998; 38:G125–G129.
40. Chen CL, Lin CF, Chiang CW, Jan MS, Lin YS. Lithium inhibits ceramide- and etopo-side-induced protein phosphatase 2A methylation, Bcl-2 dephosphorylation, caspase-2 activation, and apoptosis. Mol Pharmacol. 2006; 70:510–517. [PubMed: 16682503]
41. Chen H, Xia M, Lin M, Yang H, Kuhlenkamp J, Li T, Sodir NM, Chen YH, Josef-Lenz H, Laird PW, Clarke S, Mato JM, Lu SC. Role of methionine adenosyltransferase 2A and *S*-adenosylmethionine in mitogen-induced growth of human colon cancer cells. Gastroenterology. 2007; 133:207–218. [PubMed: 17631143]
42. Chen L, Zeng Y, Yang HP, Lee TD, French SW, Corrales FJ, García-Trevijano ER, Avila MA, Mato JM, Lu SC. Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. FASEB J. 2004 10.1096/fj.03-1204fje.
43. Chen YM, Shiu JY, Tzeng SJ, Shih LS, Chen YJ, Lui WY, Chen PH. Characterization of glycine-*N*-methyltransferase-gene expression in human hepatocellular carcinoma. Int J Cancer. 1998; 75:787–93. [PubMed: 9495250]
44. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell. 2000; 103:263–271. [PubMed: 11057899]
45. Chiang PK. Biological effects of inhibitors of *S*-adenosylhomocysteine hydrolase. Pharmacol Ther. 1998; 77:115–134. [PubMed: 9578320]
46. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, McCann PP. *S*-Adenosylmethionine and methylation. FASEB J. 1996; 10:471–480. [PubMed: 8647346]
47. Chou JY, Ruppert S, Shelly LL, Pan C. Isolation and characterization of mouse hepatocyte lines carrying a lethal albino deletion. J Biol Chem. 1991; 266:5716–5722. [PubMed: 1848557]
48. Clarke SG. Inhibition of mammalian protein methyltransferases by 5′-methylthioadenosine (MTA): a mechanism of action of dietary SAMe? The Enzymes. 2006; 24:467–493.
49. Coleman WB, Tsongalis GJ. Multiple mechanisms account for genomic instability and molecular mutation in neoplastic transformation. Clin Chem. 1995; 41:644–657. [PubMed: 7729041]
50. Cornforth JW, Reichard SA, Talalay P, Carrell HL, Glusker JP. Determination of the absolute configuration at the sulfonium center of *S*-adenosylmethionine. Correlation with the absolute configuration of the diastereomeric *S*-carboxymethyl-(*S*)-methionine salt. J Am Chem Soc. 1977; 99:7292–7300. [PubMed: 915153]

51. Corrales F, Cabrero C, Pajares MA, Ortiz P, Martin-Duce A, Mato JM. Inactivation and dissociation of *S*-adenosylmethionine synthetase by modification of sulfhydryl groups and its possible occurrence in cirrhosis. Hepatology. 1990; 11:216–222. [PubMed: 2307400]
52. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science. 1996; 274:1369–1383.
53. D'Anselmi F, Cucina A, Cavallaro G, Bizzarri M, Cavallaro RA, Fuso A, Cavallaro A, Scarpa S. *S*-adenosylmethionine inhibits ubiquitin-proteasome system in vitro and on rat vascular smooth muscle cells. Protein Pept Lett. 2008; 15:58–62. [PubMed: 18221015]
54. Dante R, Anaud M, Niveleau A. Effects of 5′-deoxy-5′-methylthioadenosine on the metabolism of *S*-adenosyl methionine. Biochem Biophys Res Commun. 1983; 114:214–221. [PubMed: 6309166]
55. Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC. Elevated hepatic fatty acid oxidation, high plasma fibroblast growth factor 21, and fasting bile acids in nonalcoholic steatohepatitis. Eur J Gastroenterol Hepatol. 2011; 23:382–388. [PubMed: 21448070]
56. De La Haba G, Jamieson GA, Mudd SH, Richards HH. *S*-adenosylmethionine: the relation of configuration at the sulfonium center to enzymatic activity. J Am Chem Soc. 1959; 81:3975–3980.
57. De La Rosa J, LeGros HL Jr, Geller AM, Kotb M. Changes in the relative amount of subunits of methionine adenosyltransferase in human lymphocytes upon stimulation with a polyclonal T cell mitogen. J Biol Chem. 1992; 267:10699–10704. [PubMed: 1587846]
58. Del Pino MM, Corrales FJ, Mato JM. Hysteretic behavior of methionine adenosyltransferase. III. Methionine switches between two conformations of the enzyme with different specific activity. J Biol Chem. 2000; 275:23476–23482. [PubMed: 10811651]
59. Ding W, Mouzaki M, You H, Laird JC, Mato J, Lu SC, Rountree CB. CD133+ liver cancer stem cells from methionine adenosyltransferase 1A-deficient mice demonstrate resistance to transforming growth factor (TGF)-beta-induced apoptosis. Hepatology. 2009; 49:1277–1286. [PubMed: 19115422]
60. Dridi L, Ahmed Ouameur A, Ouellette M. High affinity *S*-adenosylmethionine plasma membrane transporter of *Leishmania* is a member of the folate biopterin transporter (FBT) family. J Biol Chem. 2010; 285:19767–19775. [PubMed: 20406813]
61. Duschene KS, Broderick JB. The antiviral protein viperin is a radical SAM enzyme. FEBS Lett. 2010; 584:1263–1267. [PubMed: 20176015]
62. Eaton JE, Silveira MG, Pardi DS, Sinakos E, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, Harnois D, Jorgensen R, Petz J, Lindor KD. High-dose ursodeoxycholic acid is associated with the development of colorectal neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Am J Gastroenterol. 2011; 106:1638–1645. [PubMed: 21556038]
63. Feld JJ, Modi AA, El-Diwany R, Rotman Y, Thomas E, Ahlenstiel G, Titerence R, Koh C, Cherepanov V, Heller T, Ghany MG, Park Y, Hoofnagle JH, Liang TJ. *S*-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. Gastroenterology. 2011; 140:830–839. [PubMed: 20854821]
64. Feo F, Garcea R, Daino L, Pascale R, Pirisi L, Frassetto S, Ruggiu ME. Early stimulation of polyamine biosynthesis during promotion by Phenobarbital of diethylnitrosamine-induced rat liver carcinogenesis. The effects of variations of the *S*-adenosyl-l-methionine cellular pool. Carcinogenesis. 1985; 6:1713–1720. [PubMed: 2866045]
65. Fernandez-Checa, J.; Lu, SC.; Ookhtens, M.; DeLeve, L.; Runnegar, M.; Yoshida, H.; Saiki, H.; Kannan, R.; Garcia-Ruiz, C.; Kuhlenkamp, JF.; Kaplowitz, N. The regulation of hepatic glutathione. In: Tavoloni, N.; Berk, PD., editors. Hepatic Anion Transport and Bile Secretion: Physiology and Pathophysiology. 2nd ed.. Raven; New York: 1993. p. 363-395.
66. Filipowicz M, Bernsmeier C, Terracciano L, Duong FH, Heim MH. *S*-adenosyl-methionine and betaine improve early virological response in chronic hepatitis C patients with previous nonresponse. PLoS One. 2010; 5:e15492. [PubMed: 21079746]
67. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem. 1990; 1:228–237. [PubMed: 15539209]

68. Finkelstein JD. Inborn errors of sulfur-containing amino acid metabolism. J Nutr. 2006; 136:1750S–1754S. [PubMed: 16702350]
69. Finkelstein JD. Metabolic regulatory properties of *S*-adenosylmethionine and *S*-adenosylhomocysteine. Clin Chem Lab Med. 2007; 45:1694–1699. [PubMed: 17963455]
70. Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, Rizzo G, Distrutti E, Shah V, Morelli A. The third gas: H2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. Hepatology. 2005; 42:539–48. [PubMed: 16108046]
71. French SW, Bardag-Gorce F, Li J, French BA, Oliva J. Mallory-Denk body pathogenesis revisited. World J Hepatol. 2010; 2:295–301. [PubMed: 21161012]
72. Frey PA, Hegeman AD, Ruzicka FJ. The radical SAM superfamily. Crit Rev Biochem Mol Biol. 2008; 43:63–88. [PubMed: 18307109]
73. Frostesjö L, Holm I, Grahn B, Page AW, Bestor TH, Heby O. Interference with DNA methyltransferase activity and genome methylation during F9 teratocarcinoma stem cell differentiation induced by polyamine depletion. J Biol Chem. 1997; 272:4359–4366. [PubMed: 9020157]
74. Fusaro G, Dasgupta P, Rastogi S, Joshi B, Chellappan S. Prohibitin induces the transcriptional activity of p53 and is exported from the nucleus upon apoptotic signaling. J Biol Chem. 2003; 278:47853–47861. [PubMed: 14500729]
75. Gadalla MM, Snyder SH. Hydrogen sulfide as a gasotransmitter. J Neurochem. 2010; 113:14–26. [PubMed: 20067586]
76. Garcia-Trevijano ER, Latasa MU, Carretero MV, Berasain C, Mato JM, Avila MA. *S*-adenosylmethionine regulates MAT1A and MAT2A gene expression in cultured rat hepatocytes: a new role for *S*-adenosylmethionine in the maintenance of the differentiated status of the liver. FASEB J. 2000; 14:2511–2518. [PubMed: 11099469]
77. García-Trevijano ER, Martínez-Chantar ML, Latasa MU, Mato JM, Avila MA. NO sensitizes rat hepatocytes to proliferation by modifying *S*-adenosylmethionine levels. Gastroenterology. 2002; 122:1355–1363. [PubMed: 11984522]
78. Gil B, Casado M, Pajares M, Boscá L, Mato JM, Martín-Sanz P, Alvarez L. Differential expression pattern of methionine adenosyltransferase isoenzymes during rat liver development. Hepatology. 1996; 24:876–881. [PubMed: 8855191]
79. Gil B, Pajares MA, Mato JM, Alvarez L. Glucocorticoid regulation of hepatic *S*-adenosylmethionine synthetase gene expression. Endocrinology. 1997; 138:1251–1258. [PubMed: 9048633]
80. Glass JI, Assad-Garcia N, Alperovich N, Yooseph S, Lewis MR, Hutchinson CA 3rd, Smith HO, Venter JC. Essential genes of a minimal bacterium. Proc Natl Acad Sci USA. 2006; 103:425–430. [PubMed: 16407165]
81. Graham DE, Bock CL, Schalk-Hihi C, Lu ZJ, Markham GD. Identification of a highly diverged class of *S*-adenosylmethionine synthetases in the Archea. J Biol Chem. 2000; 275:4005–4059.
82. Grubbs R, Vugrek O, Deisch J, Wagner C, Stabler S, Allen R, Barić I, Rados M, Mudd SH. *S*-adenosylhomocysteine hydrolase deficiency: two siblings with fetal hydrops and fatal outcomes. J Inherit Metab Dis. 2010; 33:705–713. [PubMed: 20852937]
83. Halim AB, LeGros L, Geller A, Kotb M. Expression and functional interaction of the catalytic and regulatory subunits of human methionine adenosyltransferase in mammalian cells. J Biol Chem. 1999; 274:29720–29725. [PubMed: 10514445]
84. Hardie DG. Sensing of energy and nutrients by AMP-activated protein kinase. Am J Clin Nutr. 2011; 93:891S–896S. [PubMed: 21325438]
85. Hardy, M.; Coulter, I.; Morton, SC.; Favreau, J.; Venuturupalli, S.; Chiappelli, F.; Rossi, F.; Orshansky, G.; Jungvig, LK.; Roth, EA.; Suttorp, MJ.; Shekelle, P. S-Adenosyl-L-Methionine for Treatment of Depression, Osteoarthritis, and Liver Disease. Agency for Healthcare Research and Quality; Rockville, MD: Oct. 2002 Evidence Reports/Technology Assessments, No. 64.
86. Heby O. DNA methylation and polyamines in embryonic development and cancer. Int J Dev Biol. 1995; 39:737–757. [PubMed: 8645558]

87. Hegazi MF, Borchardt RT, Schowen RL. Letter: SN2-like transition state for methyl transfer catalyzed by catechol-*O*-methyl-transferase. J Am Chem Soc. 1976; 98:3048–3049. [PubMed: 1262638]

88. Hevia H, Varela-Rey M, Corrales FJ, Berasain C, Martinez-Chantar ML, Latasa MU, Lu SC, Mato JM, García-Trevijano ER, Avila MA. 5′-Methylthioadenosine modulates the inflammatory response to endotoxin in mice and in rat hepatocytes. Hepatology. 2004; 39:1088–1098. [PubMed: 15057913]

89. Hibasami H, Borchardt RT, Chen SY, Coward JK, Pegg AE. Studies of inhibition of rat spermidine synthase and spermine synthase. Biochem J. 1980; 187:419–428. [PubMed: 7396856]

90. Hoffman DR, Marion DW, Cornatzer WE, Duerre JA. *S*-Adenosylmethionine and *S*-adenosylhomocysteine metabolism in isolated rat liver. Effects of l-methionine, l-homocysteine, and adenosine. J Biol Chem. 1980; 255:10822–10827. [PubMed: 7430157]

91. Hoffman JL. Chromatographic analysis of the chiral and covalent instability of *S*-adenosyl-l-methionine. Biochemistry. 1986; 25:4444–4449. [PubMed: 3530324]

92. Horikawa S, Ozasa H, Ota K, Tsukada K. Immunohistochemical analysis of rat methionine adenosyltransferase isozymes in developmental liver. FEBS Lett. 1993; 330:307–311. [PubMed: 8375501]

93. Horikawa S, Tsukada K. Molecular cloning and developmental expression of a human kidney methionine adenosyltransferase. FEBS Lett. 1992; 312:37–41. [PubMed: 1426236]

94. Horowitz JH, Rypins EB, Henderson JM, Heymsfield SB, Moffitt SD, Bain RP, Chawla RK, Bleier JC, Rudman D. Evidence for impairment of transsulfuration pathway in cirrhosis. Gastroenterology. 1981; 81:668–675. [PubMed: 7262512]

95. Hote PT, Sahoo R, Jani TS, Ghare SS, Chen T, Joshi-Barve S, McClain CJ, Barve SS. Ethanol inhibits methionine adenosyltransferase II activity and *S*-adenosylmethionine biosynthesis and enhances caspase-3-dependent cell death in T lymphocytes: relevance to alcohol-induced immunosuppression. J Nutr Biochem. 2008; 19:384–391. [PubMed: 17869084]

96. Huang ZZ, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. Am J Physiol Gastrointest Liver Physiol. 1998; 294:G14–G21.

97. Huang ZZ, Mato JM, Kanel G, Lu SC. Differential effect of thioacetamide on hepatic methionine adenosyltransferase expression. Hepatology. 1999; 29:1471–1468. [PubMed: 10216131]

98. Iglesias Ara A, Xia M, Ramani K, Mato JM, Lu SC. *S*-Adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation. Hepatology. 2008; 47:1655–1666. [PubMed: 18393372]

99. Ikeda R, Nishida T, Watanabe F, Shimizu-Saito K, Asahina K, Horikawa S, Teraoka H. Involvement of CCAAT/enhancer binding protein-b (C/EBP-b) in epigenetic regulation of mouse methionine adenosyltransferase 1A gene expression. Int J Biochem Cell Biol. 2008; 40:1956–1969. [PubMed: 18346930]

100. Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M. Cystathionine gamma-lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. J Biol Chem. 2010; 285:26358–26368. [PubMed: 20566639]

101. Jani TS, Gobejishvili L, Hote PT, Barve AS, Joshi-Barve S, Kharebava G, Suttles J, Chen T, McClain CJ, Barve S. Inhibition of methionine adenosyltransferase II induces FasL expression, Fas-DISC formation and caspase-8-dependent apoptotic death in T leukemic cells. Cell Res. 2009; 19:358–369. [PubMed: 19048023]

102. Jensen D, Chaikoff IL, Tarver H. The ethionine-induced fatty liver: dosage, prevention, and structural specificity. J Biol Chem. 1951; 192:395–403. [PubMed: 14917687]

103. Kadariya Y, Yin B, Tang B, Shinton SA, Quinlivan EP, Hua X, Klein-Szanto A, Al-Saleem TI, Bassing CH, Hardy RR, Kruger WD. Mice heterozygous for germ-line mutations in methylthioadenosine phosphorylase (MTAP) die prematurely of T-cell lymphoma. Cancer Res. 2009; 69:5961–5969. [PubMed: 19567676]

104. Kabil O, Banerjee R. Redox biochemistry of hydrogen sulfide. J Biol Chem. 2010; 285:21903–21907. [PubMed: 20448039]

105. Kalhan SC, Edmison J, Marczewski S, Dasarathy S, Gruca LL, Bennett C, Duenas C, Lopez R. Methionine and protein metabolism in non-alcoholic steatohepatitis: evidence for lower rate of transmethylation of methionine. Clin Sci. 2011; 121:179–189. [PubMed: 21446920]

106. Kasashima K, Sumitani M, Satoh M, Endo H. Human prohibitin 1 maintains the organization and stability of the mitochondrial nucleoids. Exp Cell Res. 2008; 314:988–996. [PubMed: 18258228]

107. Katoh Y, Ikura T, Hoshikawa Y, Tashiro S, Ito T, Ohta M, Kera Y, Noda T, Igarashi K. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoproteins. Mol Cell. 2011; 41:554–566. [PubMed: 21362551]

108. Kee K, Foster BA, Merali S, Kramer DL, Hensen ML, Diegelman P, Kisiel N, Vujcic S, Mazurchuk RV, Porter CW. Activated polyamine catabolism depletes acetyl-CoA pools and suppresses prostate tumor growth in TRAMP mice. J Biol Chem. 2004; 279:40076–40083. [PubMed: 15252047]

109. Kee K, Vujcic S, Merali S, Diegelman P, Kisiel N, Powell CT, Kramer DL, Porter CW. Metabolic and antiproliferative consequences of activated polyamine catabolism in LNCaP prostate carcinoma cells. J Biol Chem. 2004; 279:27050–27058. [PubMed: 15096507]

110. Kinsell LW, Harper HA, Barton HC, Michaels GD, Weiss HA. Rate of disappearance from plasma of intravenously administered methionine in patients with liver damage. Science. 1947; 106:589–594.

111. Kirovski G, Stevens AP, Czech B, Dettmer K, Weiss TS, Wild P, Hartmann A, Bosser-hoff AK, Oefner PJ, Hellerbrand C. Down-regulation of methylthioadenosine phosphorylase (MTAP) induces progression of hepatocellular carcinoma via accumulation of 5′-deoxy-5′-methylthioadenosine (MTA). Am J Pathol. 2011; 178:1145–1152. [PubMed: 21356366]

112. Knappe J, Schmitt T. A novel reaction of *S*-adenosyl-l-methionine correlated with the activation of pyruvate formate-lyase. Biochem Biophys Res Commun. 1976; 71:1110–1117. [PubMed: 971302]

113. Ko K, Iglesias-Ara A, French BA, French SW, Ramani K, Tomasi ML, Lozano JJ, Oh P, He L, Stiles BL, Li TWH, Yang HP, Martínez-Chantar ML, Mato JM, Lu SC. Liver-specific deletion of prohibitin 1 results in spontaneous liver injury, fibrosis and hepatocellular carcinoma in mice. Hepatology. 2010; 52:2096–2108. [PubMed: 20890892]

114. Ko K, Yang HP, Noureddin M, Iglesia-Ara A, Xia M, Wagner C, Luka Z, Mato JM, Lu SC. Changes in *S*-adenosylmethionine and glutathione homeostasis during endotoxemia in mice. Lab Invest. 2008; 88:1121–1129. [PubMed: 18695670]

115. Koonen DP, Jacobs RL, Febbraio M, Young ME, Soltys CL, Ong H, Vance DE, Dyck JR. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. Diabetes. 2007; 56:2863–2871. [PubMed: 17728375]

116. Korendyaseva TK, Martinov MV, Dudchenko AM, Vitvitsky VM. Distribution of methionine between cells and incubation medium in suspension of rat hepatocytes. Amino Acids. 2010; 39:1281–1289. [PubMed: 20309593]

117. Kotb M, Kredich NM. Methionine adenosyltransferase from human lymphocytes. Purification and characterization. J Biol Chem. 1985; 260:3923–3930. [PubMed: 3980460]

118. Kotb M, Mudd SH, Mato JM, Geller AM, Kredich NM, Chou JY, Cantoni GL. Consensus nomenclature for the mammalian methionine adenosyltransferase genes and gene products. Trends Genet. 1997; 13:51–52. [PubMed: 9055605]

119. Kujubu DA, Stimmel JB, Law RE, Herschman HR, Clarke S. Early responses of PC-12 cells to NGF and EGF: effect of K252a and 5′-methylthioadenosine on gene expression and membrane protein methylation. J Neurosci Res. 1993; 36:58–65. [PubMed: 8230321]

120. Kumar D, Tandon RK. Use of ursodeoxycholic acid in liver disease. J Gastro Hepatol. 2001; 16:3–14.

121. Kumar M, Zhao X, Wang XW. Molecular carcinogenesis of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: one step closer to personalized medicine? Cell Biosci. 2011; 1:5. [PubMed: 21711594]

122. Latasa MU, Boukaba A, García-Trevijano ER, Torres L, Rodríguez JL, Caballería J, Lu SC, López-Rodas G, Franco L, Mato JM, Avila MA. Hepatocyte growth factor induces MAT2A

expression and histone acetylation in rat hepatocytes. Role in liver regeneration. FASEB J. 2001; 10:1096.

123. Lee TD, Sadda ME, Mendler MH, Bottiglieri T, Kanel G, Mato JM, Lu SC. Abnormal hepatic methionine and GSH metabolism in patients with alcoholic hepatitis. Alcoholism Clin Exp Res. 2004; 28:173–181.
124. LeGros LH Jr, Halim AB, Chamberlin ME, Geller A, Kotb M. Regulation of the human MAT2b gene encoding the regulatory β subunit of methionine adenosyltransferase, MAT II. J Biol Chem. 2001; 276:24918–24924. [PubMed: 11337507]
125. LeGros LH Jr, Halim AB, Geller A, Kotb M. Cloning, expression, functional characterization of the b regulatory subunit of human methionine adenosyltransferase (MAT II). J Biol Chem. 2000; 275:2359–2366. [PubMed: 10644686]
126. Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci USA. 1999; 196:7473–7478. [PubMed: 10377439]
127. Leulliot N, Quevillon-Cheruel S, Sorel I, de La Sierra-Gallay IL, Collinet B, Graille M, Blondeau K, Bettache N, Poupon A, Janin J, van Tilbeurgh H. Structure of protein phosphatase methyltransferase 1 (PPM1), a leucine carboxyl methyltransferase involved in the regulation of protein phosphatase 2A activity. J Biol Chem. 2004; 279:8351–8358. [PubMed: 14660564]
128. Levine YC, Li GK, Michel T. Agonist-modulated regulation of AMP-activated protein kinase in endothelial cells: evidence for a AMP→Rac→Akt→eNOS pathway. J Biol Chem. 2007; 282:20351–20364. [PubMed: 17519230]
129. Li J, Ramani K, Sun Z, Zee C, Grant EG, Yang HP, Xia M, Oh P, Ko K, Mato JM, Lu SC. Forced expression of methionine adenosyltransferase 1A in human hepatoma cells suppresses in vivo tumorigenesis in mice. Am J Pathol. 2010; 176:2456–2466. [PubMed: 20363925]
130. Li TWH, Yang HP, Peng H, Xia M, Mato JM, Lu SC. Effects of *S*-adenosylmethionine and methylthioadenosine on inflammation-induced colon cancer in mice. Carcinogenesis. In press.
131. Li TWH, Zhang Q, Oh P, Xia M, Chen H, Bemanian S, Lastra N, Circ M, Moyer MP, Mato JM, Aw TY, Lu SC. *S*-Adenosylmethionine and methylthioadenosine inhibit cellular FLICE inhibitory protein expression and induce apoptosis in colon cancer cells. Mol Pharmacol. 2009; 76:192–200. [PubMed: 19372210]
132. Li Y, Na K, Lee HJ, Lee EY, Paik YK. Contribution of sams-1 and pmt-1 to lipid homoeostasis in adult *Caenorhabditis elegans*. J Biochem. 2011; 149:529–538. [PubMed: 21389045]
133. Liao YJ, Liu SP, Lee CM, Yen CH, Chuang PC, Chen CY, Tsai TF, Huang SF, Lee YH, Chen YM. Characterization of a glycine *N*-methyltransferase gene knockout mouse model for hepatocellular carcinoma: implications of the gender disparity in liver cancer susceptibility. Int J Cancer. 2009; 124:816–826. [PubMed: 19035462]
134. Liau MC, Chang CF, Belanger L, Grenier A. Correlation of isozyme patterns of methionine adenosyltransferase with fetal stages and pathological states of the liver. Cancer Res. 1979; 39:162–169. [PubMed: 761186]
135. Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel *S*-adenosyl-l-methionine:arsenic(III) methyltransferase form rat liver cytosol. J Biol Chem. 2002; 277:10795–10803. [PubMed: 11790780]
136. Lin YW, Chuang SM, Yang JL. ERK1/2 achieves sustained activation by stimulating MAPK phosphatase-1 degradation via the ubiquitin-proteasome pathway. J Biol Chem. 2003; 278:21534–21541. [PubMed: 12676937]
137. Liu Q, Liu L, Zhao Y, Zhang J, Wang D, Chen J, He Y, Wu J, Zhang Z, Liu Z. Hypoxia induces genomic DNA demethylation through the activation of HIF-1a and transcriptional upregulation of MAT2A in hepatoma cells. Mol Cancer Ther. 2011; 10:1113–1123. [PubMed: 21460102]
138. Loenen WA. *S*-adenosylmethionine: jack of all trades and master of everything? Biochem Soc Trans. 2006; 34:330–333. [PubMed: 16545107]
139. Lombardi B, Pani P, Schlunk FF. Choline-deficiency fatty liver: impaired release of hepatic triglycerides. J Lipid Res. 1968; 9:437–446. [PubMed: 5725875]

140. Lombardini JB, Chou TC, Talalay P. Regulatory properties of adenosine triphosphate-l-methionine *S*-adenosyltransferase of rat liver. Biochem J. 1973; 135:43–57. [PubMed: 4590992]
141. Lombardini JB, Sufrin JR. Chemotherapeutic potential of methionine analogue inhibitors of tumor-derived methionine adenosyltransferases. Biochem Pharmacol. 1983; 32:489–495. [PubMed: 6847699]
142. Lu SC. Regulation of glutathione synthesis. Mol Asp Med. 2009; 30:42–59.
143. Lu SC. Where are we in the chemoprevention of hepatocellular carcinoma? Hepatology. 2010; 51:734–736. [PubMed: 20198627]
144. Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci USA. 2001; 98:5560–5565. [PubMed: 11320206]
145. Lu SC, Huang ZZ, Yang HP, Mato JM, Avila MA, Tsukamoto H. Changes in methionine adenosyltransferase and *S*-adenosylmethionine homeostasis in the alcoholic rat liver. Am J Physiol Gastrointest Liver Physiol. 2000; 279:G178–G185. [PubMed: 10898761]
146. Lu SC, Mato JM. *S*-adenosylmethionine in cell growth, apoptosis and liver cancer. J Gastroenterol Hepatol. 2008; 23:S73–S77. [PubMed: 18336669]
147. Lu SC, Ramani K, Ou XP, Lin M, Yu V, Ko K, Park R, Bottiglieri T, Tsukamoto H, Kanel G, French S, Mato JM, Moat R, Grant E. *S*-adenosylmethionine in the chemoprevention and treatment of hepatocellular carcinoma in a rat model. Hepatology. 2009; 50:462–471. [PubMed: 19444874]
148. Luka Z, Capdevila A, Mato JM, Wagner C. A glycine N-methyltransferase knockout mouse model for human with deficiency of this enzyme. Transgenic Res. 2006; 15:393–397. [PubMed: 16779654]
149. Luka Z, Mudd SH, Wagner C. Glycine *N*-methyltransferase and regulation of *S*-adenosylmethionine levels. J Biol Chem. 2009; 284:22507–22511. [PubMed: 19483083]
150. Marí M, Colell A, Morales A, Pañeda C, Varela-Nieto I, Garía-Ruiz C, Fernández-Checa JC. Acidic sphingomyelinase downregulates the liver-specific methionine adenosyltransferase 1A, contributing to tumor necrosis factor-induced lethal hepatitis. J Clin Invest. 2004; 113:895–904. [PubMed: 15067322]
151. Markham GD, Pajares MA. Structure-function relationships in methionine adenosyltransferases. Cell Mol Life Sci. 2009; 66:636–648. [PubMed: 18953685]
152. Marobbio CM, Agrimi G, Lasorsa FM, Palmieri F. Identification and functional reconstitution of yeast mitochondrial carrier for *S*-adenosylmethionine. EMBO J. 2003; 22:5975–82. [PubMed: 14609944]
153. Marongiu F, Doratiotto S, Montisci S, Pani P, Laconi E. Liver repopulation and carcinogenesis: two sides of the same coin? Am J Pathol. 2008; 172:857–864. [PubMed: 18321999]
154. Martínez-Chantar ML, Corrales FJ, Martínez-Cruz A, García-Trevijano ER, Huang ZZ, Chen LX, Kanel G, Avila MA, Mato JM, Lu SC. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. FASEB J. 2002; 10:1096.
155. Martínez-Chantar ML, Garcia-Trevijano ER, Latasa MU, Martin-Duce A, Fortes P, Caballeria J, Avila MA, Mato JM. Methionine adenosyltransferase II β subunit gene expression provides a proliferative advantage in human hepatoma. Gastroenterology. 2003; 124:940–948. [PubMed: 12671891]
156. Martínez-Chantar ML, Latasa MU, Varela-Rey M, Lu SC, García-Trevijano E, Mato JM, Avila MA. l-Methionine availability regulates the expression of methionine adenosyltransferase 2A gene in human hepatocarcinoma cells. J Biol Chem. 2003; 278:19885–19890. [PubMed: 12660248]
157. Martinez-Chantar ML, Lu SC, Mato JM, Luka Z, Wagner C, French BA, French SW. The role of stem cells/progenitor cells in liver carcinogenesis in glycine *N*-methyltransferase deficient mice. Exp Mol Pathol. 2010; 88:234–237. [PubMed: 20080087]
158. Martínez-Chantar ML, Vázquez-Chantada M, Ariz U, Martínez N, Varela M, Luka Z, Capdevila A, Rodríguez J, Aransay AM, Matthiesen R, Yang H, Calvisi DF, Esteller M, Fraga M, Lu SC,

Wagner C, Mato JM. Loss of the glycine *N*-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology. 2008; 47:1191–1199. [PubMed: 18318442]

159. Martínez-Chantar ML, Vázquez-Chantada M, Garnacho M, Latasa MU, Varela-Rey M, Dotor J, Santamaria M, Martínez-Cruz LA, Parada LA, Lu SC, Mato JM. *S*-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. Gastroenterology. 2006; 131:223–232. [PubMed: 16831604]
160. Martínez-López N, Varela-Rey M, Fernández-Ramos D, Woodhoo A, Vázquez-Chantada M, Embade N, Espinosa-Hevia L, Bustamante FJ, Parada LA, Rodriguez MS, Lu SC, Mato JM, Martínez-Chantar ML. Activation of LKB1-Akt pathway independent of phosphoinositide 3-kinase plays a critical role in the proliferation of hepatocellular carcinoma from nonalcoholic steatohepatitis. Hepatology. 2010; 52:1621–1631. [PubMed: 20815019]
161. Martinov MV, Vitvitsky VM, Banerjee R, Ataullakhanov FI. The logic of the hepatic methionine metabolic cycle. Biochim Biophys Acta. 2010; 1804:89–96. [PubMed: 19833238]
162. Mato JM, Alvarez L, Ortiz P, Pajares MA. *S*-adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol Ther. 1997; 73:265–280. [PubMed: 9175157]
163. Mato JM, Cámara J, Fernández de Paz J, Caballería L, Coll S, Caballero A, García-Buey L, Beltrán J, Benita V, Caballería J, Solà R, Moreno-Otero R, Barrao F, Martín-Duce A, Correa JA, Parés A, Barrao E, García-Magaz I, Puerta JL, Moreno J, Boissard G, Ortiz P, Rodés J. *S*-adenosylmethionine in alcoholic liver cirrhosis: a randomized placebo-controlled, double-blind, multicentre trial. J Hepatol. 1999; 30:1081–1089. [PubMed: 10406187]
164. Mato JM, Lu SC. Role of *S*-adenosyl-l-methionine in liver health and injury. Hepatology. 2007; 45:1306–1312. [PubMed: 17464973]
165. Mato, JM.; Lu, SC. *S*-adenosylmethionine. In: Coates, PM.; Blackman, MR.; Levine, M.; Moss, J.; White, JD., editors. Encyclopedia of Dietary Supplements. 2nd ed.. Dekker; New York: 2010. p. 1-6.
166. Mato JM, Martínez-Chantar ML, Lu SC. Methionine metabolism and liver disease. Annu Rev Nutr. 2008; 28:273–93. [PubMed: 18331185]
167. Matthews RP, Lorent K, Mañoral-Mobias R, Huang Y, Gong W, Murray IV, Blair IA, Pack M. TNFalpha-dependent hepatic steatosis and liver degeneration caused by mutation of zebrafish *S*-adenosylhomocysteine hydrolase. Development. 2009; 136:865–875. [PubMed: 19201949]
168. McClung JK, Danner DB, Stewart DA, Smith JR, Schneider EL, Lumpkin CK, Dell'Orco RT, Nuell MJ. Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. Biochem Biophys Res Commun. 1989; 164:1316–1322. [PubMed: 2480116]
169. Medici V, Virata MC, Peerson JM, Stabler SP, French SW, Gregory JF 3rd, Albanese A, Bowlus CL, Devaraj S, Panacek EA, Richards JR, Halsted CH. *S*-adenosyl-l-methionine treatment for alcoholic liver disease: a double-blinded, randomized, placebo-controlled trial. Alcohol Clin Exp Res. 2011; 35:1960–1965. [PubMed: 22044287]
170. Merali S, Clarkson AB Jr. *S*-adenosylmethionine and *Pneumocystis*. FEMS Microbiol Lett. 2004; 237:179–186. [PubMed: 15321660]
171. Merali S, Vargas D, Frankli M, Clarkson AB Jr. *S*-adenosylmethionine and *Pneumocystis carinii*. J Biol Chem. 2000; 275:14958–14963. [PubMed: 10809741]
172. Merkwirth C, Langer T. Prohibitin function within the mitochondria: essential roles for cell proliferation and cristae morphogenesis. Biochim Biophys Acta. 2009; 1793:27–32. [PubMed: 18558096]
173. Michalopoulos GK, DeFrances MC. Liver regeneration. Science. 1997; 276:60–66. [PubMed: 9082986]
174. Mingorance J, Alvarez L, Sánchez-Góngora E, Mato JM, Pajares MA. Site-directed mutagenesis of rat liver *S*-adenosylmethionine synthetase. Biochem J. 1996; 315:761–766. [PubMed: 8645155]
175. Mishra S, Murphy LC, Murphy LJ. The Prohibitins: emerging roles in diverse functions. J Cell Mol Med. 2006; 10:353–363. [PubMed: 16796804]
176. Misteli T. RNA splicing: what has phosphorylation got to do with it? Curr Biol. 1999; 9:R198–200. [PubMed: 10209090]

177. Morgan TR. Chemoprevention of hepatocellular carcinoma in chronic hepatitis C. Recent Results Cancer Res. 2011; 188:85–99. [PubMed: 21253791]

178. Mudd SH, Cerone R, Schiaffino MC, Fantasia AR, Minniti G, Caruso U, Lorini R, Watkins D, Matiaszuk N, Rosenblatt DS, Schwahn B, Rozen R, LeGros L, Kotb M, Capdevila A, Luka Z, Finkelstein JD, Tangerman A, Stabler SP, Allen RH, Wagner C. Glycine *N*-methyltransferase deficiency: a novel inborn error causing persistent isolated hypermethioninaemia. J Inherit Metab Dis. 2001; 24:448–464. [PubMed: 11596649]

179. Mudd SH, Poole JR. Labile methyl balances for normal humans on various dietary regimens. Metabolism. 1975; 24:721–735. [PubMed: 1128236]

180. Nelson JC. *S*-adenosyl methionine (SAMe) augmentation in major depressive disorder. Am J Psychiatry. 2010; 167:889–891. [PubMed: 20693465]

181. Nieto N, Cederbaum AI. *S*-adenosylmethionine blocks collagen I production by preventing transforming growth factor-β induction of the COL1A2 promoter. J Biol Chem. 2005; 280:30963–30974. [PubMed: 15983038]

182. Nijtmans LG, de Jong L, Artal Sanz M, Coates PJ, Berden JA, Back JW, Muijsers AO, van der Spek H, Grivell LA. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J. 2000; 19:2444–2451. [PubMed: 10835343]

183. Nijtmans LGJ, Sanz MA, Grivell LA, Coates PJ. The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing and degenerative disease. Cell Mol Life Sci. 2002; 59:143–155. [PubMed: 11852914]

184. Nguyen LN, Furuya MH, Wolfraim LA, Nguyen AP, Holdren MS, Campbell JS, Knight B, Yeoh GC, Fausto N, Parks WT. Transforming growth factor-beta differentially regulates oval cell and hepatocyte proliferation. Hepatology. 2007; 45:31–41. [PubMed: 17187411]

185. Obolenskaya M, Schulze-Specking A, Plaumann B, Frenzer K, Freudenberg N, Decker K. Nitric oxide production by cells isolated from regenerating rat liver. Biochem Biophys Res Commun. 1994; 204:1305–1311. [PubMed: 7980609]

186. Okada G, Teraoka H, Tsukada K. Multiple species of mammalian methionine adenosyltransferase. Partial purification and characterization. Biochemistry. 1981; 20:934–940. [PubMed: 7213623]

187. Okada G, Watanabe Y, Tsukada K. Changes in patterns of methionine adenosyltransferase in fetal and postnatal rat liver. Cancer Res. 1980; 40:2895–2897. [PubMed: 7388839]

188. Osna NA, White RL, Donohue TM Jr, Beard MR, Tuma DJ, Kharbanda KK. Impaired methylation as a novel mechanism for proteasome suppression in liver cells. Biochem Biophys Res Commun. 2010; 391:1291–1296. [PubMed: 20026058]

189. Pajares MA, Corrales F, Ochoa P, Mato JM. The role of cysteine-150 in the structure and activity of rat liver *S*-adenosyl-l-methionine synthetase. Biochem J. 1991; 274:225–229. [PubMed: 2001237]

190. Pajares MA, Duran C, Corrales F, Pliego M, Mato JM. Modulation of rat liver *S*-adenosylmethionine synthetase activity by glutathione. J Biol Chem. 1992; 267:17598–17605. [PubMed: 1517209]

191. Pajares MA, Duran C, Corrales F, Mato JM. Protein kinase C phosphorylation of rat liver methionine adenosyltransferase: dissociation and product of an active monomer. Biochem J. 1994; 303:949–955. [PubMed: 7980467]

192. Pajula RL, Raina A. Methylthioadenosine, a potent inhibitor of spermine synthase from bovine brain. FEBS Lett. 1979; 99:343–345. [PubMed: 428559]

193. Pajula RL, Raina A, Eloranta T. Polyamine synthesis in mammalian tissues. Isolation and characterization of spermine synthase from bovine brain. Eur J Biochem. 1979; 101:619–626. [PubMed: 520313]

194. Pañeda C, Gorospe I, Herrera B, Nakamura T, Fabregat I, Varela-Nieto I. Liver cell proliferation requires methionine adenosyltransferase 2A mRNA up-regulation. Hepatology. 2002; 35:1381–1391. [PubMed: 12029623]

195. Papakostas GI, Mischoulon D, Shyu I, Alpert JE, Fava M. *S*-adenosyl methionine (SAMe) augmentation of serotonin reuptake inhibitors for antidepressant nonresponders with major

depressive disorder: a double-blind, randomized clinical trial. Am J Psychiatry. 2010; 167:942–948. [PubMed: 20595412]

196. Pascale RM, Simile MM, De Miglio MR, Nufris A, Daino L, Seddaiu MA, Rao PM, Rajalakshmi S, Sarma DS, Feo F. Chemoprevention by *S*-adenosyl-l-methionine of rat liver carcinogenesis initiated by 1,2-dimethylhydrazine and promoted by orotic acid. Carcinogenesis. 1995; 16:427–430. [PubMed: 7859377]
197. Pascale R, Simile MM, Ruggiu ME, Seddaiu MA, Satta G, Sequenza MJ, Daino L, Vannini MG, Lai P, Feo F. Reversal by 5-azacytidine of the *S*-adenosyl-l-methionine-induced inhibition of the development of putative preneoplastic foci in rat liver carcinogenesis. Cancer Lett. 1991; 56:259–265. [PubMed: 1708694]
198. Pascale RM, Simile MM, Satta G, Seddaiu MA, Daino L, Pinna G, Vinci MA, Gaspa L, Feo F. Comparative effects of L-methionine, *S*-adenosyl-l-methionine and 5′-methylthioadenosine on the growth of preneoplastic lesions and DNA methylation in rat liver during the early stages of hepatocarcinogenesis. Anticancer Res. 1991; 11:1617–1624. [PubMed: 1684097]
199. Pegg AE. Spermidine/spermine-N(1)-acetyltransferase: a key metabolic regulator. Am J Physiol Endocrinol Metab. 2008; 294:E995–E1010. [PubMed: 18349109]
200. Pegg AE, Wiliams-Ashman HG. Phosphate-stimulated breakdown of 5′-methylthioadenosine by rat ventral prostate. Biochem J. 1969; 115:241–247. [PubMed: 5378381]
201. Perez-Leal O, Merali S. Regulation of polyamine metabolism by translational control. Amino Acids. 2012; 42:611–617. [PubMed: 21811825]
202. Perez-Leal O, Moncada C, Clarkson AB, Merali S. Pneumocystis *S*-adenosylmethionine transport: a potential drug target. Am J Respir Cell Mol Biol. 2011; 45:1142–1146. [PubMed: 21642588]
203. Perez-Mato I, Castro C, Ruiz FA, Corrales FJ, Mato JM. Methionine adenosyltransferase *S*-nitrosylation is regulated by the basic and acidic amino acids surrounding the target thiol. J Biol Chem. 1999; 274:17075–17079. [PubMed: 10358060]
204. Petrossian TC, Clarke SG. Uncovering the human methyltransferasome. Mol Cell Proteomics. 2011 Epub 2010 Oct 7.
205. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fuji C. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest. 2008; 118:560–570. 2008. [PubMed: 18219394]
206. Porter DH, Cook RJ, Wagner C. Enzymatic properties of dimethylglycine dehydrogenase and sarcosine dehydrogenase from rat liver. Arch Biochem Biophys. 1985; 243:396–407. [PubMed: 2417560]
207. Prudova A, Bauman Z, Braun A, Vitvitsky V, Lu SC, Banerjee R. AdoMet regulation of cystathionine β-synthase: relevance to oxidative stress in liver disease. Proc Natl Acad Sci USA. 2006; 103:6489–6494. [PubMed: 16614071]
208. Ragione FD, Pegg AE. Effect of analogous of 5′-methylthioadenosine on cellular metabolism. Biochem J. 1983; 210:429–435. [PubMed: 6407475]
209. Rai RM, Lee FYJ, Rosen A, Yang SQ, Lin HZ, Koteish A, Liew FY, Zaragoza C, Lowenstein C, Diehl AM. Impaired liver regeneration in inducible nitric oxide synthase-deficient mice. Proc Natl Acad Sci USA. 1998; 95:13829–13834. [PubMed: 9811886]
210. Raina A, Hyvonen T, Eloranta T, Voutilainen M, Samejima K, Yamanoha B. Inhibition of the synthesis of polyamines and macromolecules by 5′-methylthioadenosine and 5′-alkylthiotubercidins in BHK21 cells. Biochem J. 1984; 219:991–1000. [PubMed: 6743257]
211. Ramana CV, Boldogh I, Izumi T, Mitra S. Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. Proc Natl Acad Sci USA. 1998; 95:5061–5066. [PubMed: 9560228]
212. Ramani K, Mato JM, Lu SC. Role of methionine adenosyltransferase genes in hepatocarcinogenesis. Cancers. 2011; 3:1480–1497.
213. Ramani K, Yang HP, Kuhlenkamp J, Tomasi ML, Tsukamoto H, Mato JM, Lu SC. Changes in methionine adenosyltransferase and *S*-adenosylmethionine during hepatic stellate cell activation. Hepatology. 2010; 51:986–995. [PubMed: 20043323]

214. Ramani K, Yang HP, Xia M, Iglesias Ara A, Mato JM, Lu SC. Leptin's mitogenic effect in human liver cancer cells requires induction of both methionine adenosyltransferase 2A and 2β. Hepatology. 2008; 47:521–531. [PubMed: 18041713]
215. Rambaldi A, Gluud C. *S*-adenosyl-l-methionine for alcoholic liver disease. Cochrane Database Syst Rev. 2001; 4:CD002235. [PubMed: 11687153]
216. Rao MS, Reddy JK. Peroxisomal beta-oxidation and steatohepatitis. Semin Liver Dis. 2001; 21:43–55. [PubMed: 11296696]
217. Reytor E, Perez-Miguelsanz J, Alvarez L, Perez-Sala D, Pajares MA. Conformational signals in the C-terminal domain of methionine adenosyltransferase I/III determine its nucleocytoplasmic distribution. FASEB J. 2009; 23:3347–3360. [PubMed: 19497982]
218. Richards HH, Chiang PK, Cantoni Adenosylhomocysteine hydrolase GL. Crystallization of the purified enzyme and its properties. J Biol Chem. 1978; 253:4476–4480. [PubMed: 659427]
219. Rodríguez JL, Boukaba A, Sandoval J, Georgieva EI, Latasa MU, García-Trevijano ER, Serviddio G, Nakamura T, Avila MA, Sastre J, Torres L, Mato JM, López-Rodas G. Transcription of the MAT2A gene, coding for methionine adenosyltransferase, is up-regulated by E2F and Sp1 at a chromatin level during proliferation of liver cells. Int J Biochem Cell Biol. 2007; 39:842–850. [PubMed: 17317269]
220. Rouillon A, Surdin-Kerjan Y, Thomas D. Transport of sulfonium compounds. Characterization of the s-adenosylmethionine and s-methylmethionine permeases from the yeast *Saccharomyces cerevisiae*. J Biol Chem. 1999; 274:28096–28105. [PubMed: 10497160]
221. Rountree CB, Senadheera S, Mato JM, Crooks GM, Lu SC. Expansion of liver cancer stem cells during aging in methionine adenosyltransferase 1A deficient mice. Hepatology. 2008; 47:1288–1297. [PubMed: 18167064]
222. Ruiz F, Corrales FJ, Miqueo C, Mato JM. Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by *S*-nitrosylation. Hepatology. 1998; 128:1051–1057. [PubMed: 9755242]
223. Russmann S, Junker E, Lauterburg BH. Remethylation and transsulfuration of methionine in cirrhosis: studies with l-[H3-methyl-1-C]methionine. Hepatology. 2002; 36:1190–1196. [PubMed: 12395329]
224. Rutjes AW, Nüesch E, Reichenbach S, Jüni P. *S*-adenosylmethionine for osteoarthritis of the knee or hip. Cochrane Database Syst Rev. 2009; 4:CD007321. [PubMed: 19821403]
225. Sakata SF, Shelly LL, Ruppert S, Schutz G, Chou JY. Cloning and expression of murine methionine adenosyltransferase. J Biol Chem. 1993; 268:13978–13986. [PubMed: 8314764]
226. Sánchez-Góngora E, Ruiz F, Mingorance J, An W, Corrales FJ, Mato JM. Interaction of liver methionine adenosyltransferase with hydroxyl radical. FASEB J. 1997; 11:1013–1019. [PubMed: 9337154]
227. Santamaria E, Avila MA, Latasa MU, Rubio A, Martin-Duce A, Lu SC, Mato JM, Corrales FJ. Functional proteomics of nonalcoholic steatohepatitis: mitochondrial proteins as targets of *S*-adenosylmethionine. Proc Natl Acad Sci USA. 2003; 100:3065–3070. [PubMed: 12631701]
228. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci USA. 2008; 105:7034–7039. [PubMed: 18467494]
229. Scavetta RD, Thomas CB, Walsh MA, Szegedi S, Jooachimiak A, Gumport RI, Churchill MEA. Structure of *Rsr*I methyltransferase, a member of the *N*6-adenine β class of DNA methyltransferases. Nucleic Acids Res. 2000; 28:3950–3961. [PubMed: 11024175]
230. Sell S. Cellular origin of hepatocellular carcinomas. Semin Cell Dev Biol. 2002; 13:419–424. [PubMed: 12468242]
231. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, DePinho RA, Montminy M, Cantley LC. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science. 2005; 310:1642–1646. [PubMed: 16308421]
232. Silveira MG, Lindor KD. Treatment of primary biliary cirrhosis: therapy with choleretic and immunosuppressive agents. Clin Liver Dis. 2008; 12:425–443. [PubMed: 18456189]
233. Singal R, Ginder GD. DNA methylation. Blood. 1999; 93:4059–4070. [PubMed: 10361102]

234. Skordi E, Claus SP, Martin FP, Cloarec O, Lindberg J, Schuppe-Kolstinen I, Holmes E, Nicholson JK. Analysis of time-related metabolic fluctuations induced by ethionine in the rat. J Proteome Res. 2007; 6:4572–4581. [PubMed: 17966971]

235. Steglich G, Neupert W, Langer T. Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol Cell Biol. 1999; 19:3435–3442. [PubMed: 10207067]

236. Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q, Koonin EV, Davis RW. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science. 1998; 282:754–759. [PubMed: 9784136]

237. Stipanuk MH. Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr. 2004; 24:539–577. [PubMed: 15189131]

238. Sullivan DM, Hoffman J. Fractionation and kinetic properties of rat liver and kidney methionine adenosyltransferase isozymes. Biochemistry. 1983; 22:1636–1641. [PubMed: 6849873]

239. Teng YW, Mehedint MG, Garrow TA, Zeisel SH. Deletion of betaine-homocysteine *S*-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. J Biol Chem. 2011; 286:36258–36267. [PubMed: 21878621]

240. Tobeña R, Horikawa S, Calvo V, Alemany S. Interleukin-2 induces γ-*S*-adenosyl-l-methionine synthetase gene expression during T-lymphocyte activation. Biochem J. 1996; 319:929–933. [PubMed: 8921001]

241. Tomasi ML, Iglesias Ara A, Yang H, Ramani K, Feo F, Pascale MR, Martínez-Chantar ML, Mato JM, Lu SC. *S*-adenosylmethionine regulates apurinic/apyrimidinic endonuclease 1 stability: implication in hepatocarcinogenesis. Gastroenterology. 2009; 136:1025–1036. [PubMed: 18983843]

242. Tomasi ML, Li TWH, Li M, Mato JM, Lu SC. Inhibition of methionine adenosyltransferase 1A transcription by coding region methylation. J Cell Physiol. 2012; 227:1583–1591. [PubMed: 21678410]

243. Tomasi ML, Ramani K, Lopitz-Otsoa F, Rodríguez MS, Li TWH, Ko K, Yang HP, Bardag-Gorce F, Iglesias-Ara A, Feo F, Pascale MR, Mato JM, Lu SC. *S*-adenosylmethionine regulates dual-specificity MAPK phosphatase expression in mouse and human hepatocytes. Hepatology. 2010; 51:2152–2161. [PubMed: 20196119]

244. Tonks NK, Neel BG. From form to function: signaling by protein tyrosine phosphatases. Cell. 1996; 87:365–368. [PubMed: 8898190]

245. Torres L, Avila MA, Carretero MV, Latasa MU, Caballeria J, López-Rodas G, Boukaba A, Lu SC, Franco L, Mato JM. Liver-specific methionine adenosyltransferase MAT1A gene expression is associated to a specific pattern of promoter methylation and histone acetylation. Implications for MAT1A silencing during transformation. FASEB J. 2000; 14:95–102. [PubMed: 10627284]

246. Tseng TL, Shih YP, Huang YC, Wang CK, Chen PH, Chang JG, Yeh KT, Chen YM, Buetow KH. Genotypic and phenotypic characterization of a putative tumor susceptibility gene, GNMT, in liver cancer. Cancer Res. 2003; 63:647–654. [PubMed: 12566309]

247. Tsukamoto HC, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. FASEB J. 2001; 15:1335–1349. [PubMed: 11387231]

248. Ubagai T, Lei KJ, Huang S, Mudd SH, Levy HL, Chou Y. Molecular mechanisms of an inborn error of methionine pathway. J Clin Invest. 1995; 96:1943–1947. [PubMed: 7560086]

249. Uno K, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Imai J, Hasegawa Y, Gao J, Kaneko K, Iwasaki H, Ishihara H, Sasano H, Inukai K, Mizuguchi H, Asano T, Shiota M, Nakazato M, Oka Y. Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. Science. 2006; 312:1656–1659. [PubMed: 16778057]

250. Varela-Rey M, Beraza N, Lu SC, Mato JM, Martínez-Chantar ML. Role of AMPK in the control of hepatocyte priming and proliferation during liver regeneration. Exp Biol Med. 2011; 236:402–408.

251. Varela-Rey M, Fernández-Ramos D, Martínez-López N, Embade N, Gómez-Santos L, Beraza N, Vázquez-Chantada M, Rodríguez J, Luka Z, Wagner C, Lu SC, Martínez-Chantar ML, Mato JM. Impaired liver regeneration in mice lacking glycine *N*-methyltransferase. Hepatology. 2009; 50:443–452. [PubMed: 19582817]

252. Varela-Rey M, Martínez-López N, Fernández-Ramos D, Embade N, Calvisi DF, Woodhoo A, Rodríguez J, Fraga MF, Julve J, Rodríguez-Millán E, Frades I, Torres L, Luka Z, Wagner C, Esteller M, Lu SC, Martínez-Chantar ML, Mato JM. Fatty liver and fibrosis in glycine *N*-methyltransferase knockout mice is prevented by nicotinamide. Hepatology. 2010; 52:105–114. [PubMed: 20578266]

253. Vázquez-Chantada M, Ariz U, Varela-Rey M, Embade N, Martínez-Lopez N, Fernández-Ramos D, Gómez-Santos L, Lamas S, Lu SC, Martínez-Chantar ML, Mato JM. Evidence for LKB1/AMP-activated protein kinase/endothelial nitric oxide synthase cascade regulated by hepatocyte growth factor, *S*-adenosylmethionine, and nitric oxide in hepatocyte proliferation. Hepatology. 2009; 49:608–617. [PubMed: 19177591]

254. Vázquez-Chantada M, Fernández D, Embade N, Woodhoo A, Martinez N, Varela-Rey M, Luka Z, Wagner C, Caballería J, Gorospe M, Lu SC, Mato JM, Martínez-Chantar ML. HuR/Methylated-HuR and AUF1 regulate the expression of methionine adenosyltransferase during liver proliferation, differentiation and carcinogenesis. Gastroenterology. 2010; 138:1943–1953. [PubMed: 20102719]

255. Vinci CR, Clarke SG. Recognition of age-damaged (*R,S*)-adenosyl-l-methionine by two methyltransferases in the yeast *Sacharomyces cerevisiae*. J Biol Chem. 2007; 282:8604–8612. [PubMed: 17264075]

256. Vinci CR, Clarke SG. Homocysteine methyltransferases Mht1 and Sam4 prevent the accumulation of age-damaged (*R,S*)-AdoMet in the yeast *Saccharomyces cerevisiae*. J Biol Chem. 2010; 285:20526–20531. [PubMed: 20421295]

257. Vinci CR, Clarke SG. Yeast, plants, worms and flies use a methyltransferase to metabolize age-damaged (*R,S*)-AdoMet, but what do mammals do? Rejuvenation Res. 2010; 13:362–364. [PubMed: 20370499]

258. Vugrek O, Beluzić R, Nakić N, Mudd SH. *S*-adenosylhomocysteine hydrolase (AHCY) deficiency: two novel mutations with lethal outcome. Hum Mutat. 2009; 30:E555–565. [PubMed: 19177456]

259. Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, Shioda T, Hansen M, Yang F, Niebergall LJ, Vance DE, Tzoneva M, Hart AC, Näär AM. A conserved SREBP1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell. 2011; 147:840–852. [PubMed: 22035958]

260. Wang S, Fusaro G, Padmanabhan J, Chellappan SP. Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression. Oncogene. 2002; 21:8388–8396. [PubMed: 12466959]

261. Watson WH, Zhao Y, Chawla RK. *S*-adenosylmethionine attenuates the lipopolysaccharide-induced expression of the gene for tumour necrosis factor alpha. Biochem J. 1999; 342:21–25. [PubMed: 10432295]

262. Wong FW, Chan WY, Lee SS. Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. Toxicol Appl Pharmacol. 1998; 153:109–118. [PubMed: 9875305]

263. Woodard RW, Tsai MD, Floss HG, Crooks PA, Coward JK. Stereochemical course of the transmethylation catalyzed by catechol *O*-methyltransferase. J Biol Chem. 1980; 255:9124–9127. [PubMed: 6997310]

264. Woodcock DM, Adams JK, Allan RG, Cooper IA. Effect of several inhibitors of enzymatic DNA methylation on the in vivo methylation of different classes of DNA sequences in a cultured human cell line. Nucleic Acids Res. 1983; 11:489–499. [PubMed: 6186987]

265. Wu SE, Huskey WP, Borchardt RT, Schowne REL. Chiral instability at sulfur of *S*-adenosylmethionine. Biochemistry. 1983; 22:2828–2832. [PubMed: 6871165]

266. Wu SM, Huang YH, Lu YH, Chien LF, Yeh CT, Tsai MM, Liao CH, Chen WJ, Liao CJ, Cheng WL, Lin KH. Thyroid hormone receptor-mediated regulation of the methionine adenosyltransferase 1 gene is associated with cell invasion in hepatoma cell lines. Cell Mol Life Sci. 2010; 67:1831–1843. [PubMed: 20146079]

267. Xia M, Chen Y, Wang LC, Zandi E, Yang H, Bemanian S, Martinez-Chantar ML, Mato JM, Lu SC. Novel function and intracellular localization of methionine adenosyltransferase 2beta splicing variants. J Biol Chem. 2010; 285:20015–20021. [PubMed: 20421296]

268. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA. 1997; 94:1441–1446. [PubMed: 9037072]

269. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science. 2008; 322:587–590. [PubMed: 18948540]

270. Yang HP, Huang ZZ, Zeng ZH, Chen CJ, Selby RR, Lu SC. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. Am J Physiol Gastrointest Liver Physiol. 2001; 280:G184–G190. [PubMed: 11208539]

271. Yang HP, Huang ZZ, Zeng ZH, Chen CJ, Wang JH, Lu SC. The role of c-Myb and Sp1 in the up-regulation of methionine adenosyltransferase 2A gene expression in human hepatocellular carcinoma. FASEB J. 2001; 15:1507–1516. [PubMed: 11427482]

272. Yang HP, Iglesias Ara A, Magilnick N, Xia M, Ramani K, Chen H, Lee TD, Mato JM, Lu SC. Expression pattern, regulation and function of methionine adenosyltransferase 2β alternative splicing variants in hepatoma cells. Gastroenterology. 2008; 134:281–291. [PubMed: 18045590]

273. Yang HP, Ko K, Xia M, Li TWH, Oh P, Li J, Lu SC. Induction of avian musculoaponeurotic fibrosarcoma proteins by toxic bile acid inhibits expression of GSH synthetic enzymes and contributes to cholestatic liver injury in mice. Hepatology. 2010; 51:1291–1301. [PubMed: 20146260]

274. Yang HP, Ramani K, Xia M, Ko KS, Li TWH, Oh P, Li J, Lu SC. Dysregulation of glutathione synthesis during cholestasis in mice: molecular mechanisms and therapeutic implications. Hepatology. 2009; 49:1982–1991. [PubMed: 19399914]

275. Yang HP, Sadda MR, Li M, Zeng Y, Chen L, Bae W, Ou X, Runnegar MT, Mato JM, Lu SC. *S*-adenosylmethionine and its metabolite induce apoptosis in HepG2 cells: role of protein phosphatase 1 and Bcl-x(S). Hepatology. 2004; 40:221–231. [PubMed: 15239106]

276. Yang HP, Sadda MR, Yu V, Zeng Y, Lee TD, Ou XP, Chen LX, Lu SC. Induction of human methionine adenosyltransferase 2A expression by tumor necrosis factor alpha: role of NF-κB and AP-1. J Biol Chem. 2003; 278:50887–50896. [PubMed: 14530285]

277. Zeng ZH, Huang ZZ, Chen CJ, Yang HP, Mao Z, Lu SC. Molecular cloning and functional characterization of the 5′-flanking region of human methionine adenosyltransferase 1A gene. Biochem J. 2000; 346:475–482. [PubMed: 10677369]

278. Zhang W, Wagner BJ, Ehrenman K, Schaefer AW, DeMaria CT, Crater D, DeHaven K, Long L, Brewer G. Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. Mol Cell Biol. 1993; 13:7652–7665. [PubMed: 8246982]

279. Zhuge J, Cederbaum AI. Depletion of *S*-adenosyl-l-methionine with cycloleucine potentiates cytochrome *P*450 2E1 toxicity in primary rat hepatocytes. Arch Biochem Biophys. 2007; 486:177–185. [PubMed: 17640612]

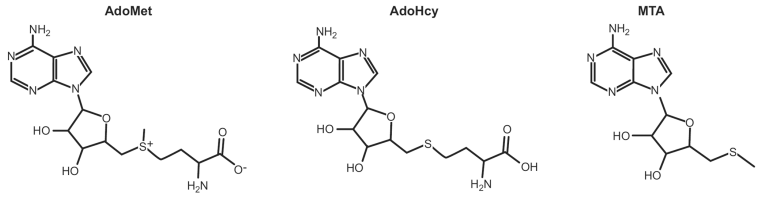

FIGURE 1.
Structure of *S*-adenosyl-L-methionine (AdoMet), *S*-adenosyl-L-homocysteine (AdoHcy), and 5′-methylthioadenosine (MTA).

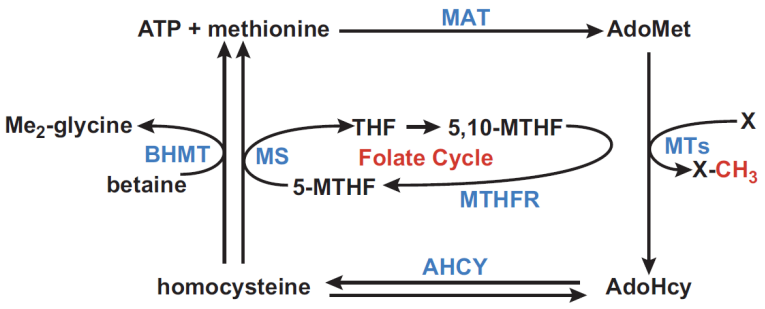

FIGURE 2.

Methionine metabolism: transmethylation pathway. Methionine adenosyltransferase (MAT) catalyzes the conversion of methionine and ATP into AdoMet. In transmethylation reactions, AdoMet donates its methyl group to a large variety of acceptor molecules (X) in reactions catalyzed by methyl transferases (MTs). AdoMet-dependent methylation reactions yield AdoHcy as a byproduct. AdoHcy cellular content is regulated by the enzyme AdoHcy hydrolase (AHCY), which reversibly cleaves this molecule into adenosine and homocysteine. Remethylation of homocysteine to form methionine occurs by two enzymes: methionine synthase (MS), which requires normal levels of folate and vitamin B_{12}, and betaine homocysteine methyltransferase (BHMT), which requires betaine, a metabolite of choline. MS-catalyzed homocysteine remethylation requires 5-methyltetrahydrofolate (5-MTHF), which is derived from 5,10-methylenetetrahydrofolate (5,10-MTHF) in a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR). 5-MTHF is then converted to tetrahydrofolate (THF) as it donates its methyl group and THF is converted to 5,10-MTHF to complete the folate cycle.

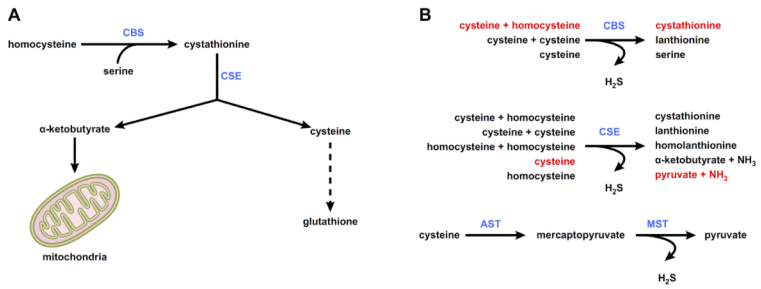

FIGURE 3.

Methionine metabolism: transsulfuration and hydrogen sulfide synthesis. *A*: the transsulfuration pathway links AdoMet to cysteine biosynthesis. Here homocysteine is converted to cysteine (the rate-limiting precursor for glutathione) via a two-step enzymatic process catalyzed by cystathionine β-synthase (CBS) and cystathionase (CSE), both requiring vitamin B_6. α-Ketobutyrate, the other product of cystathionine cleavage, is further metabolized by the mitochondria through the Kreb's cycle. *B*: hydrogen sulfide synthesis. Although the classical role of CBS and CSE is to generate cystathionine and cysteine, respectively, these two enzymes catalyze multiple hydrogen sulfide (H_2S)-generating reactions using cysteine and homocysteine as substrates. The predominant H_2S-generating reactions are shown in red. A third pathway uses aspartate aminotransferase (AST) to yield mercaptopyruvate, which is further converted into H_2S in a reaction catalyzed by mercaptopyruvate sulfurtransferase (MST). The function of this pathway is primarily catabolic.

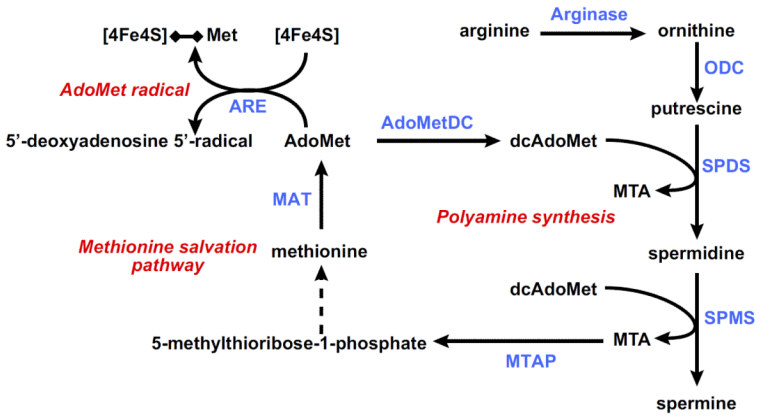

FIGURE 4.

Methionine metabolism: polyamine synthesis, methionine salvation pathway, and AdoMet radical reactions. To synthesize polyamines, AdoMet needs first to be decarboxylated, a reaction catalyzed by the enzyme AdoMet decarboxylase (AdoMetDC), to form decarboxylated AdoMet (dcAdoMet). The predominant polyamines in mammalian cells are spermidine (SPD) and spermine (SPM). These polyamines are made by sequential addition of aminopropyl groups from dcAdoMet, yielding 5′-methylthioadenosine (MTA) as a byproduct. SPD synthase (SPDS) catalyzes the transfer of the first aminopropyl group from dcAdoMet to putrescine to form SPD and MTA, whereas SPM synthase (SPMS) catalyzes the transfer of the second aminopropyl group to SPD to form SPM and a second molecule of MTA. MTA is metabolized through the methionine salvation pathway to regenerate AdoMet. The first reaction in this pathway is the cleavage of MTA by the enzyme MTA phosphorylase (MTAP) yielding adenine and 5-methylthioribose-1-phosphate, which is further metabolized to methionine and adenine to AMP. AdoMet may be also converted to 5′-deoxyadenosyl 5′-radical by a large family of AdoMet radical enzymes (ARE) and initiate a variety of radical chemical reactions. These enzymes share a CX_3CX_2C motif forming a characteristic [4Fe-4S] cluster. This [4Fe-4S] cluster binds AdoMet catalyzing its reductive cleavage to generate [4Fe-4S]-methionine and a 5′-deoxyadenosyl 5′-radical. ODC, ornithine decarboxylase; MAT, methionine adenosyltransferase.

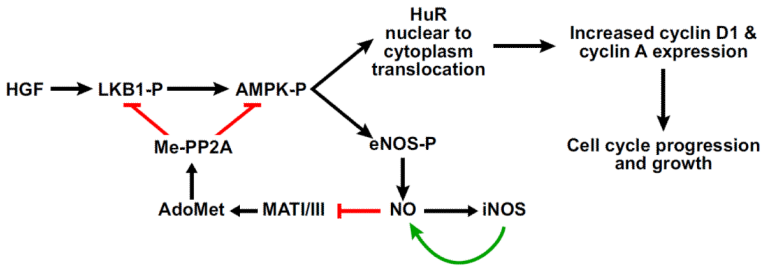

FIGURE 5.

HGF-LKB1/AMPK signaling and AdoMet regulation. Although AMPK and LKB1 are traditionally thought of as metabolic tumor suppressors, they induce hepatocyte proliferation, via HuR cytoplasm translocation and stabilization of cyclin D1 and cyclin A expression, following hepatocyte growth factor (HGF) stimulation, and AdoMet can block this process. Activated AMPK induces the phosphorylation and activation of endothelial nitric oxide synthase (eNOS) leading to the generation of nitric oxide (NO). NO thus generated activates inducible NOS (iNOS) leading to a further increase in NO and the inactivation of MAT I/III. As a result, AdoMet content decreases releasing the inhibitory effect that this molecule exerts on LKB1 and AMPK phosphorylation via methylation and activation of phosphoprotein phosphatases 2A (Me-PP2A).

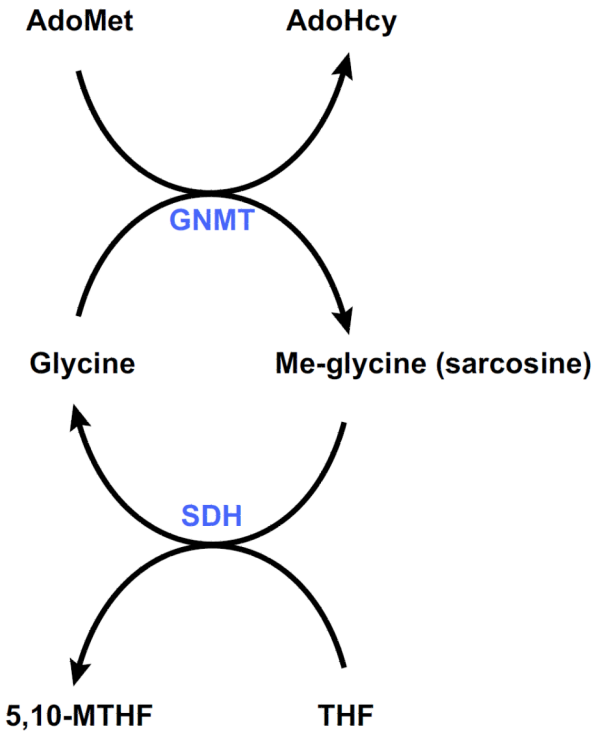

FIGURE 6.
AdoMet catabolism. Excess AdoMet generated in the liver after a protein-rich meal is rapidly eliminated by the enzyme glycine *N*-methyltransferase (GNMT), which catalyzes the synthesis of methyl-glycine (sarcosine) from glycine. Sarcosine dehydrogenase (SDH) is a flavoprotein that catalyzes the oxidative demethylation of sarcosine to glycine. In this reaction, tetrahydrofolate (THF) is converted to 5,10-methyllenetrahydrofolate (5,10-MTHF).

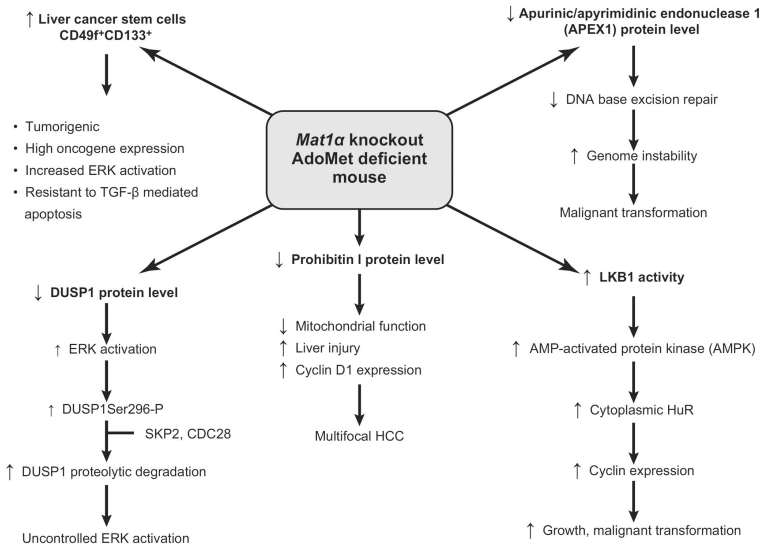

FIGURE 7.

Signaling pathways leading to HCC in AdoMet-deficient *Mat1a* KO mice. Several abnormal pathways have been identified in *Mat1a* KO mice that can contribute to hepatocellular carcinoma (HCC) formation: *1*) a fall in apurinic/apyrimidinic endonuclease 1 (APEX1) activity, which leads to a reduction in DNA base excision repair, genome instability, and malignant transformation; *2*) an increase in LKB1 activity, which induces the activation of AMPK and an increased translocation of HuR from the nucleus to the cytoplasm leading to the stabilization of several cyclin mRNAs and enhanced growth; *3*) a reduction in prohibitin 1 (PHB1) content, which leads to impaired mitochondrial function and liver injury, increased cyclin D1 expression, and multifocal HCC; *4*) a decrease in dual-specificity phosphatase 1 (DUSP1), which leads to uncontrolled ERK activation, a hallmark of HCC; and *5*) expansion of tumorigenic oval stem cells positive for CD133, a cancer stem cell marker. See text for details.

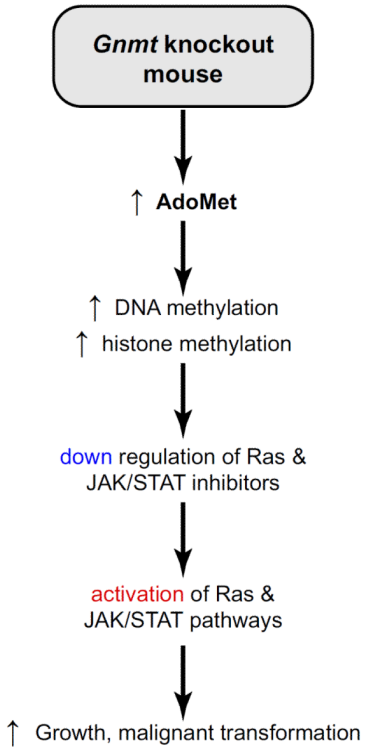

FIGURE 8.

Signaling pathways leading to HCC in *Gnmt* KO mice. Loss of GNMT activity induces an abnormal accumulation of hepatic AdoMet leading to a major epigenetic alteration in gene expression regulation. These epigenetic changes include the hypermethylation of *Rassf1* and *Socs2* promoters, as well as the trimethylation of histone 3 lysine 27 associated with these genes. As a consequence, the expression of *Rassf1* and *Socs2*, as well as of other inhibitors of Ras and JAK/STAT signaling pathways, is downregulated. This leads to the activation of Ras and JAK/STAT signaling pathways, to abnormal growth, and to malignant transformation.

Table 1

Properties and regulation of mammalian MAT genes

MAT Gene	Protein Product	Isoenzymes	K_m for Methionine	Inhibition by	Expression Pattern in Healthy Liver	HCC	Reference Nos.
MAT1A	α1	MATI-(α1)4	23 μM-1 mM	ROS, NO (activity)	High	Low	26, 78, 117, 164
		MATIII-(α1)2		215 μM-7 mM	High	Low	
MAT2A	α2	MATII-Structure varies in tissue-specific manner	4–10 μM	AdoMet (activity and mRNA level)	Low	High	26, 78, 117, 164, 214
MAT2B	β (V1)	Interacts with MATII	Lowers K_m of MATII	AdoMet (mRNA level)	Low	High	117, 155, 214, 272
	V2	Interacts with MATII	?	?	Low	High	

See text for details.